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CONTENTS

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EXO-ERYTHROCYTIC SCHIZOGONY OF A MALARIA PARASITE IN THE HIMALAYAN FLYING SQUIRREL, *PETAURISTA INORNATUS* (GEOFFREY)

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(Received July 29, 1960)

ABSTRACT

E.E. phase in the liver of the Himalayan flying squirrel (*Petaurista inornatus*) comprised of (1) merocysts and (2) group of small cysts. Occurrence of exo-erythrocytic schizogony in the form of group of small cysts has not been met with in any known malarial infection so far. Since the intermediate host has not so far been discovered for this group of malaria parasites it is considered proper to refer the parasite to the genus *Plasmodium* for the present.

INTRODUCTION

The finding of groups of small cysts, in the liver of the Himalayan flying squirrels showing malarial infection in their blood (Ray 1949), seems to have created some confusion in the literature on the exo-erythrocytic schizogony of this malaria parasite.

For example, in the liver of the baboon (*Papio papio*) Garnham and Pick (1952) came across abnormal merocysts of large size, the interior of which, as Garnham has stated, 'was thrown into convolutions with trabeculae dividing the cyst into compartments or they were " multicystic " like Ray's parasites'. Again, Edeson (1953), in his description of the presumed exo-erythrocytic schizonts of *Plasmodium knowlesi* in the liver of Malayan monkey (*Macacus irus*), encountered 'cystic bodies' with finger-like processes projecting in all directions. In his sections passing through the poles, he showed that the central mass had disappeared, leaving only the sections of finger-like processes which then had an appearance very much similar to what he calls the 'daughter cysts', described by Ray (1949). It is thus clear that the true nature of groups of small cysts (besides the merocyst of 'Kochi' type), in this flying squirrel, has not been correctly appreciated, and that there is a tendency to believe that these small cysts were but parts of convolutions arising from a central mass or band of protoplasm. With a view to removing any misunderstanding on the subject, it is proposed to give a detailed description of the forms of E.E. stages of the malaria parasite, in the flying squirrel.

SMALL CYSTS IN GROUPS

In a preliminary note, Ray (1949) gave a short description of these small cysts, as seen in smears and sections of the liver. In freshly teased out

preparation with saline, these bodies appeared as transparent discs with clear thin limiting membrane enclosing a finely granular mass of protoplasm. On fixing and staining, the mass of protoplasm was found to be studded with nuclei (*see* Ray 1949, Pl. IX, Figs. 1 and 2). In sections of the liver these cysts were always found in groups, and never occurred singly (Ray 1949, Pl. X, Fig. 4).

The shape of the small cysts, as seen in smear preparation (Pl. I, Fig. 6), varied from round, oval, elliptical to irregular forms measuring 50 μ to 132·50 μ along their broadest diameter. Merocysts in the liver or spleen of the flying squirrel never showed lobulation, trabeculation or exfoliation of the proto-plasmic band. The small cysts, therefore, were not the product of any such phenomenon. In smear preparations, besides showing a definite cell wall, the merozoites, within these small independent and complete cysts, showed different stages of maturity. Some parenchyma cells of the liver showed young schizonts (Pl. I, Figs. 1, 2 and 3) in their cytoplasm. In others, the cytoplasm of the parasite was broken up into small islands, each containing a number of nuclei (Pl. I, Fig. 8). At a later stage, when the merozoites were fully formed, this demarcation disappeared, and small round or oval merozoites were seen embedded in a hyaline ground substance (Pl. I, Fig. 7 and Pl. II, Fig. 9). These small cysts were unlike fragments of merocyst des-cribed by Garnham (1948, Fig. 6) in that they possessed a distinct cyst wall. In serial sections there was no evidence of any central mass of protoplasm. but instead, small cysts ran from one end to the other. No central stalk or pedicel was discernible to indicate their unicentric origin. In many instances the central area showed spent up cysts with shrivelled up walls and escaping merozoites (Pl. I, Fig. 5). This naturally leads one to think that small cysts had their own individuality and were not the products of fragmentation of any large cyst or mass of protoplasm. Although there is no direct evidence, but it is quite likely that large numbers of parenchyma cells were simultaneously infected, which resulted in the formation of a group of small cysts. Invariably each small cyst, as it progressed towards maturity, showed a central vacuole (Pl. II, Fig. 9).

Host tissue reaction round groups of small cyst was absent. Had it been the breakdown product of a merocyst, one would expect to find a zonal reaction as is evident round the merocyst of *Kochi*, or even round the merocyst present in the same tissue of the flying squirrel.

Merocyst

In the preliminary note (Ray 1949) merocysts measuring 2 mm. in diameter have been described from the liver of five, spleen of two, and the lungs of one, flying squirrels. Each of these merocysts showed central vacuole filled with fluid, a peripheral band of protoplasm studded with

1   2   3   4
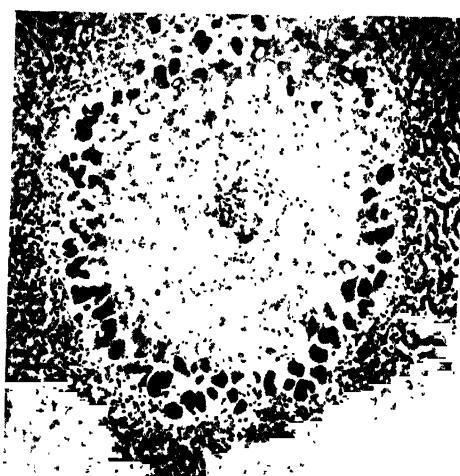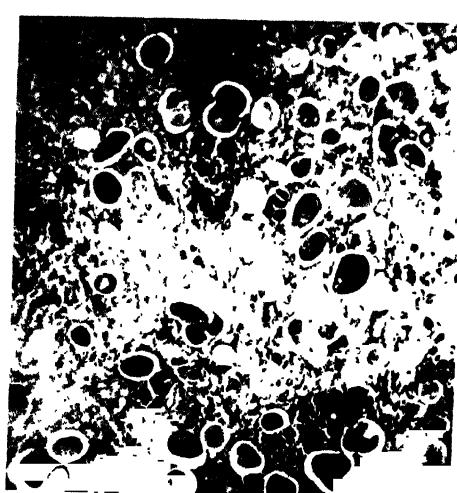
5   6

7   8
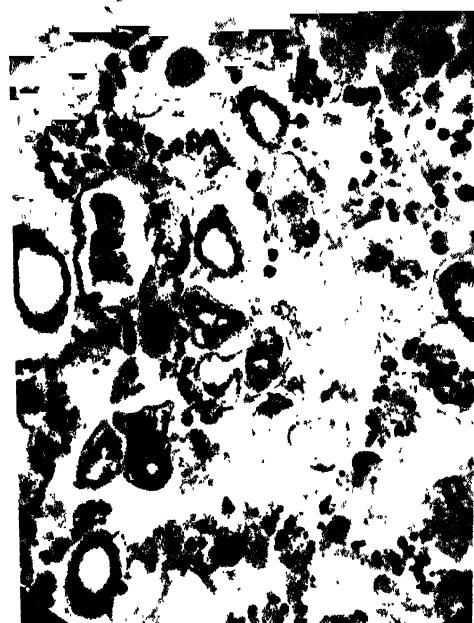
9

10

11

12

nuclei, and an outer smooth and hyaline cystic membrane. In some merocysts, however, there were a few islands of protoplasmic globules floating in the colloidal mass. This very much resembled the abnormal cyst of *H. kochi* described by Garnham and Pick (1952) in baboon.

In a preparation of merocyst of *H. kochi*,* a very thin membrane (eosin stained) was seen to be applied closely to the protoplasmic mass of the cyst. In one of the sections one nucleus was seen lying in between the membrane and the cytoplasmic mass of the merocyst. This suggested intracellular origin of merocyst, and that the thin membrane represented the remnant of the host cell.

In the merocyst of the flying squirrel, the condition was slightly different. In younger forms, the surrounding cyst wall, measuring about 15 μ in thickness, appeared as a thick unstained (some time eosin stained) homogeneous structure with several degenerated nuclei embedded in the matrix of the wall. In the older cysts, the surrounding wall appeared as double layered structure. The inner portion took up the haematoxylin stain, while the outer one either remained unstained, or took up a faint eosin stain (Pl. II, Fig. 11). Both these walls now measured only 3·75 μ to 5·0 μ in thickness.

Merocysts encountered in the spleen also showed a similar cyst wall, but the protoplasmic band here was found to be thrown into numerous folds containing numerous nuclei (Pl. II, Figs. 10 and 12). There was no marked zonal reaction round the merocysts in the spleen. This was unlike that of merocysts encountered in the liver.

Merocysts in the flying squirrel were never seen to break off and release the merozoites, while the small cysts were often seen to release the merozoites into the sinusoids of the liver.

In view of the above description, it is evident that there are two types of exo-erythrocytic schizogony in this squirrel: (1) Merocyst of *Kochi* type and (2) cluster of small cysts. It is also evident that the latter does not give rise to the former for the following reasons:

(*a*) merozoites formed within the small cysts escape, and there is no evidence of fusion of these small cysts to form a large merocyst;

(*b*) a small cyst is not seen to grow beyond a particular size, thus the possibility of one small cyst growing into a large merocyst is remote; and

(*c*) had each of these small cysts grown into a merocyst, there would have occurred a forest of merocysts at one place. This was never seen.

The question now arises, whether there are two different parasites, or there is only one parasite undergoing two types of E.E. phase. As regards the former, no assertion can be made at this stage except that the gametocytes encountered in the blood of these flying squirrels were not different from those of *Plasmodium vassali*, in other words, to *Kochi* group of parasites. No erythrocytic schizogony was encountered and, at the same time, it was not possible to establish a mixed infection.

As regards the second alternative, two types of E.E. phase were constantly encountered in all the five cases so far examined after autopsy. This was very suggestive of two types of E.E. cycle for the same parasite—a feature which is not noted for any known *Plasmodium* or *Hepatocystis*.

The parasite in this squirrel differed from *H. kochi* of *Cercopithecus kochi* as described by Garnham (1947, 1948) in the following features :

    (i) existence of a definite wall round the merocyst which is unlike the thin hyaline margin of *H. kochi*,

    (ii) occurrence of merocysts in tissues other than liver, that is, in spleen and lungs, and

    (iii) co-existence of small cysts in the liver representing a distinct E.E. phase, and being different from those represented by merocyst.

These merocysts differed from those described by Edeson (1953) in *Macacus irus* in the total absence of finger-like projection of the protoplasmic band.

DISCUSSION

In the present state of our knowledge about the systematic position of the malaria parasite in the flying squirrel, it is rather hard to refer it to the genus *Hepatocystis* or to *Plasmodium* or *Nycteria* (Garnham and Heisch 1953). It would appear that this parasite combines certain characters of all these three genera. Formation of merocyst and absence of erythrocytic schizogony indicate its affinity to *Hepatocystis*. While structurally the small cyst would appear to be of *Plasmodium* and *Nycteria* type; it differed from them by its occurrence in clusters. Occurrence of merocyst in organs other than the liver is also a feature which differentiated this parasite from *Hepatocystis*. It would appear nearer to *Nycteria* than *Plasmodium* in absence of erythrocytic schizogony.

These conclusions are, however, drawn on the assumption that merocysts and clusters of small cysts represent the life cycle of one and the same parasite. It is obvious that until the arthropod cycle of the parasite is known it would be *sub judice* to refer the organism to any one genus, and as such, it would perhaps be better to describe it as *Plasmodium* sp. for the time being.

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATES

Photomicrographs of exo-erythrocytic stages of *Plasmodium* sp. of the Himalayan flying squirrel, *Petaurista inornatus*.

PLATE 1

FIGS. 1, 2 and 3. Hepatic cells showing early schizonts. From section of liver fixed in Zenker's fluid and stained with Haematoxylin—Azure II-eosin. × 1050.

FIG. 4. Enlarged hepatic cell with growing schizont. Note the nucleus of the host cell focused on the upper surface in the centre. From a smear fixed in Schaudinn's fluid and stained with iron-haematoxylin-chromotrop 2R. × 500.

FIG. 5. Section passing through a group of small cysts in the liver. Note the central vacuolated area showing the exhausted group of small cysts from which merozoites have escaped. From section of liver fixed in Bouin's fluid and stained by Haematoxylin—Azure II-eosin. × 80.

FIG. 6. A group of small cysts. Smear made from a teased out preparation of a group of small cysts. Fixed and stained as in Fig. 4. × 160.

FIG. 7. One of the small cysts showing large number of merozoites and a definite cell wall. Smear fixed and stained as in Fig. 3. × 860.

FIG. 8. A growing small cyst showing accumulation of nuclei in small groups and a definite cell wall. Note the nucleus of the host cell on the right. Smear fixed and stained as in Fig. 4. × 860.

PLATE II

FIG. 9. A group of small cysts in section. Note a definite cell wall round each cyst. Some of the cysts are in the process of releasing the merozoites. From section of liver fixed and stained as in Fig. 5. × 240.

FIG. 10. Section of a merocyst in spleen. Fixed and stained as in Fig. 5. × 100.

FIG. 11. Section of growing merocyst in liver. Note the double layered outer membrane. Fixed and stained as in Fig. 5. × 500.

FIG. 12. A merocyst in the spleen showing trabeculated band of protoplasm studded with merozoites and a well-defined outer wall. Fixed and stained as in Fig. 5. × 500.

ON THE PROTECTIVE ACTION OF VERSENE AGAINST RADIATION DAMAGE TO GRASSHOPPER CHROMOSOMES

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ABSTRACT

The effect of treatment with versene solution before irradiation on the frequency of chromosome breakage was determined by counting the number of dicentric bridges in the first meiotic anaphase cells of the grasshopper, *Gesonula punctifrons*. In the controls (treated with 0·67 per cent saline plus 86r of X-rays), $10·18 \pm 0·27$ bridges were recorded as compared to $8·29 \pm 0·61$ per cent in the treated series (10^{-3}M versene solution in 0·67 per cent saline plus 86r of X-rays). The unirradiated versene treated group showed no bridges in 801 first anaphase cells. It was concluded that versene is a definite though feeble protector of radiation-induced chromosome breaks in our material.

INTRODUCTION

It is now generally accepted that radiation-induced chromosome breakages in animal and plant cells result from an imperfectly understood chain of reactions intervening between initial ionization and the final production of aberrations. The cells have been pre-treated with a variety of chemical substances in the hope of breaking the chain at some stage of the reactions. The successful experiments not only demonstrate the validity of the chain-reaction hypothesis and help us in understanding the intermediate events, but the results also have an important practical application. The chemicals thus discovered may be used to ensure protection to the hereditary material of animals and plants liable to be exposed to high frequency radiations.

Experiments on whole body irradiation of mice are considered to have a direct bearing on the problem of protection of human being against radiation damage and therefore they have been extensively used in the search for protective chemicals. The basis of damage and protection in these cases may after all be chromosomal and, therefore, the study of animal chromosome is likely to be of significance in this connection. But most of the chromosomal studies from this point of view have hitherto been largely confined to root tip cells of *Allium, Tradescantia, Vicia*, etc. Even here the results are contradictory.

Under the circumstances stated above, we considered it desirable to extend the studies on protective chemicals on the chromosomes of the spermatocyte cells of the grasshopper, *Gesonula punctifrons*. Our experience has shown that this material is suitable for accurate quantitative study of radiation

effects (Ray-Chaudhuri and Sarkar 1952; Ray-Chaudhuri and Pyne 1954; Ray-Chaudhuri et al. 1957; Ray-Chaudhuri and Sarkar 1958).

MATERIAL AND METHOD

The experimental material was the adult males of the grasshopper, *Gesonula punctifrons*, a semi-aquatic species which lives on the swollen petiole and succulent green leaves of water hyacinth in bogs and ponds. The dicentric bridges detected in the first meiotic anaphase divisions of the testes were taken as a type of chromosome aberration to be counted in order to indicate the effect of a chelating chemical, versene (ethelenediaminetetraacetic acid), during irradiation. These bridges, although morphologically similar to the inversion bridges, arise as a result of single chromosome breaks in the leptotene or the pre-leptotene stage (*see* Ray-Chaudhuri and Sarkar 1952; Ray-Chaudhuri and Pyne 1954).

Eight sets of experiments were carried out (Table 1) and in all experiments (except experiments I, II and III) the grasshoppers were randomly divided into two lots. Versene dissolved in 0·67 per cent saline (10^{-3}M) was injected into the abdomen of the males of one of these lots.

TABLE 1

Details of the eight sets of experiments

Experiment	Date	Number of grasshoppers examined		
		0·67 per cent saline and radiation	Versene and radiation	Versene only
I	May 1, 1958	10	11	7
II	May 22, 1958	9	12	6
III	July 16, 1958	12	13	5
IV	Aug. 8, 1958	10	13	—
V	Sept. 9, 1958	13	12	—
VI	Oct. 22, 1958	11	11	—
VII	Nov. 1, 1958	10	11	—
VIII	Nov. 11, 1958	10	12	—
Total		85	95	18

Since the efficiency of versene for chelating calcium and magnesium ions is maximum at pH values 7·5 or above, the pH value was adjusted to 7·5 by the addition of a small quantity of sodium hydroxide. The other lot was injected with 0·67 per cent saline solution. After three hours, the time for the entrance of the chelating agent into the cells as found by Wolff and Luippold (1956) in *Vicia* seeds, both the lots were put into separate muslin bags and were

then irradiated with the help of a Picker's X-ray therapy tube operated at 110 kVP and 4 mA with 1 mm. Al filtration. The grasshoppers were kept at a distance of 30 cm. from the target and were irradiated for two minutes giving a total dose of 86r as measured by a Victoreen r-meter.

Irradiated grasshoppers were transferred to jars containing fresh leaves of water hyacinth. In addition to the above two lots, in experiments I, II, and III. a third lot was injected with versene solution (10^{-3}M) and kept unirradiated with a view to observing its effect on the chromosomes, if any. The jars were transferred to a room having a constant temperature of $25°\pm1°$ C. After 28 hours, the insects were sacrificed, their testes were dissected out and fixed in alcohol acetic acid mixture (3 : 1) and ultimately stored in 70 per cent alcohol. Temporary aceto-carmine squash preparations were made from the stored material for scoring the results. First meiotic anaphase cells were looked into carefully for dicentric bridges in all the three lots.

EXPERIMENTAL RESULTS

Out of a total of 6,981 first anaphase cells. 569 dicentric bridges are observed and the results are scored from 198 grasshoppers in the eight sets of experiments designed for the purpose. In the first three sets of experiments it is observed that no bridges are formed in the unirradiated lots injected only with versene solution. The results are summarized in Table 2.

TABLE 2

Frequency of dicentric bridges in experiments I–VIII

Experi-ment	Normal saline radiation (controlled)			Versene radiation (treated)			Versene only		
	a	b	c	a	b	c	a	b	c
I	400	41	10·25	450	39	8·67	422	0	0·00
II	300	33	11·00	422	38	9·00	168	0	0·00
III	456	47	10·30	342	30	8·77	211	0	0·00
IV	320	30	9·37	283	20	7·07	.	--	
V	426	45	10·56	693	61	8·80	-	.	—
VI	425	43	10·11	300	25	8·33	--	—-	—·
VII	400	38	9·50	292	21	7·19	——	——	——
VIII	270	28	10·37	401	30	7·48	. --	—-	——
Total	2,997	305	10·18 ± 0·27	3,183	264	8·29 ± 0·61	801	0	0·00

a — number of anaphase cells examined; b = number of bridges detected; c = percentage of bridges.

It is revealed from the table that though there are variations in the percentage of bridges in different experiments, yet there is a significant difference in the frequency of dicentric bridges between the controlled and treated lots. In the controlled series a total number of 2,997 first anaphase cells and 305 bridges are obtained. thus giving a frequency of $10 \cdot 18 \pm 0 \cdot 27$ per cent of bridges. whereas in the treated series, a total number of 3,183 first anaphase cells and 264 bridges are recorded. thus giving a bridge frequency of $8 \cdot 29 \pm 0 \cdot 61$ per cent. It is also seen that in the unirradiated series. 801 first anaphase cells are examined but no bridge is recorded. Due to this negative response, this series is left out in experiments IV–VIII.

Chi-square test of homogeneity of the results in different experiments shows a value of $0 \cdot 413$ and $1 \cdot 022$ in the controlled and treated series respectively. For seven degrees of freedom the value of P is very near to one and therefore the test shows that the data are greatly homogeneous. To determine the significance of the difference in frequencies obtained in the treated and controlled lots. ' t ' test is used. It is found that the difference is significant at 1 per cent and 5 per cent levels and therefore the lower frequency of bridges found in the lot treated with versene before irradiation is not due to any sampling error. We conclude from our experiments that versene affords some protection to the meiotic chromosomes against X-radiation.

DISCUSSION

It appears that the protective effect of chelating agents against radiation-induced chromosomal aberrations has not been described so far. Our results show that versene is a definite, though weak, protector of grasshopper chromosomes. They further show that versene alone is quite ineffective in producing chromosome breakages in our material. The above results are somewhat surprising in view of the data obtained by Wolff and Luippold (1956) who have not only shown that pre-treatment with versene increases the total yield of X-ray aberrations in *Vicia* chromosomes, but also have demonstrated that treatment with even a low concentration of the chemical induces chromosome breakages in that material. Wolff and Luippold's findings are quite in accord with the work of Steffenson (1955 and 1957) who finds that when *Tradescantia paludosa* is grown in a calcium-deficient nutrient medium, the spontaneous rate of chromosome breakage is greatly increased. He also indicates that ionic depletion increases the chromosome sensitivity to X-rays. A similar viewpoint is also expressed by Mazia (1954) who proposed that chromosomes are divided into particulate units linked through bridges of divalent cations, calcium and/or magnesium and, therefore, both spontaneous and radiation-induced chromosome breakages will be sensitive to the ionic environment of the chromosomes.

The work of the above authors is not in accord with the equally convincing results of Kaufmann and McDonald (1957) who find that there is no fragmentation of the salivary gland chromosomes of *Drosophila melanogaster* when treated with versene.   Kaufmann and McDonald (1957) also do not find fragmentation in onion root tip chromosomes when treated with versene solution.   Davidson (1958) obtains similar results in the same material, but finds chromosome fragmentation in *Tradescantia* roots.

The only evidence of versene and other chelating agents being useful in protection from hazards of radiation comes from the work of Bacq, Herve, and Fischer (*see* Bacq and Alexander 1955) who have been able to show that pre-treatment with the chemical prolongs the life of mice receiving a dose of whole body irradiation sufficient to kill all the controls within a few days.

A probable mechanism of how chelating agents function as protectors comes from the suggestion of Bacq *et al.* (1958).   These authors take the view that deoxyribonuclease plays an important part in the destruction of DNA that follows irradiation.   Since DNA-ase requires magnesium-ions as co-factor for its activity (Tamm and Chargaff 1951), chelating agents, by binding magnesium-ions, render DNA-ase ineffective to a certain extent.

The same hypothesis may account for the protective action of versene in our material if we assume that DNA-ase is also important in mediating radiation-induced chromosome breakage.   Such a conclusion will find further support when positive results are obtained in animal chromosome material with other chelating agents.

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OBSERVATIONS ON THE OVARIES OF SOME INDIAN BATS

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Abstract

Some observations on the ovaries of seven species of tropical bats (two Megachiroptera and five Microchiroptera) are recorded.

The development of the follicles follows the same pattern as in most eutherian mammals. Only in *Megaderma* there is a precocious formation of the antrum by the formation of vacuoles round the zona of the ovum in the bilaminar stage of the follicle.

In all the species, the fully formed Graafian follicle has a large antrum and one or two layers of cumulus cells round the ovum. In this respect the Graafian follicles of the tropical bats differ from those of the temperate vespertilionid bats in which there is a very large cumulus composed of hypertrophied cells.

A large number of multi-ovular follicles are formed in the ovaries of *Megaderma* during early stages of the oestrous cycle. It is likely that the multi-ovular condition is reached by an abnormal division of the ovum in the follicle.

Large masses of accessory corpora lutea are formed in the ovaries of some species of bats during pregnancy.

Introduction

A study of the literature reveals that very little information is available concerning the structure of the ovaries of tropical bats. However, it is apparent that the ovaries of these animals present certain characteristic differences from the ovaries of the hibernating bats inhabiting cold climates. It is also interesting to note that there are significant differences between the hibernating bats and the tropical bats (which do not hibernate) with respect to their breeding habits. Whereas a long period of quiescence (5 to 7 months) intervenes between copulation and ovulation in the hibernating bats, copulation is followed immediately by ovulation and pregnancy in the tropical bats. It is reasonable to assume that the differences in the details of the histology of the ovaries of the bats may be in some way correlated with the differences in their breeding habits. A definite conclusion about this relationship can be arrived at provided adequate details are available regarding the ovarian structure and the breeding habits of a sufficient number of tropical and temperate bats. Since so little is yet known about the histology of the ovaries of the tropical bats, it was felt that the present paper on the structure of the ovaries of some Indian bats might be of interest and value. *

HISTORICAL

The study of the ovaries of hibernating bats has attracted the attention of workers for a long time. For a critical analysis of the earlier work on this subject, the reader is referred to the paper by Wimsatt (1944). During recent years detailed investigations on the structure of the ovaries of a few species of hibernating vespertilionid bats have been carried out by Wimsatt (1944), Sluiter and Bels (1951), Pearson et al. (1952), Sluiter et al. (1952), and Wimsatt and Kallen (1957). In the species studied by these authors the Graafian follicle, which is destined to rupture, attains its full development before the bat goes into hibernation, and remains in an almost unaltered condition until just prior to ovulation which occurs when the bat 'wakes' up several months later. During this long period which extends to several months, the Graafian follicle is characterized by the presence of a large cumulus with hypertrophied cells surrounding the ovum, and consequently the antrum is relatively small. Wimsatt and Kallen (1957) demonstrated the accumulation of glycogen within the hypertrophied cumulus cells, and interpreted this unique nature of the Graafian follicle of the hibernating bats 'as an adaptation to provide a source of energy making possible the long survival of the follicle under conditions of a drastically reduced metabolism in the hibernating animal'.

Amongst the tropical bats the structure of the ovary has been studied in recent years in *Scotophilus wroughtoni* (Gopalakrishna 1948), *Desmodus rotundus* (Wimsatt and Trapido 1952), and *Taphozous longimanus* (Gopalakrishna 1955). In all these species the Graafian follicle has a small cumulus composed of one or two layers of cells, and a large antrum. The histological picture is not unlike that of the follicle in most of the eutherian mammals. Thus the ovaries of the tropical bats so far studied appear to present a different histological picture from the ovaries of hibernating bats.

MATERIAL AND METHODS

The ovaries of the following species of bats have been studied here:— *Pteropus giganteus giganteus, Cynopterus sphinx gangeticus* (both Pteropidae), *Rhinopoma kinneari* (Rhinopomidae), *Megaderma lyra lyra* (Megadermatidae) and *Hipposideros bicolor pallidus* (Hipposideridae). In addition to these species whose ovaries have been studied for the first time, pertinent details of the histology of the ovaries of *Scotophilus wroughtoni* (Vespertilionidae) and *Taphozous longimanus* (Emballonuridae) are also included for comparison. Some aspects of the ovarian histology of both these latter species have been previously published (Gopalakrishna 1948 and 1955).

Most of the specimens had been collected through several years, and many specimens of each species were examined for the present study. The ovaries were fixed in alcoholic Bouin's fluid, dehydrated through graded alcohol,

embedded in paraffin and cut at a thickness of 8 to 10 μ. The sections were stained with Ehrlich's haematoxylin and counterstained with eosin. A few fresh specimens of each species were also collected and their ovaries were mostly subjected to the same histological technique. A few sections in each case were also stained by the periodic acid-Schiff (PAS) procedure of McManus (1946) after fixation in Rassman fluid.

OBSERVATIONS AND DISCUSSION

General comments on the breeding habits

Details of the sex-cycle of *Scotophilus wroughtoni* (Gopalakrishna 1947–1949), *Taphozous longimanus* (Gopalakrishna 1955), and *Megaderma lyra lyra* (Ramaswamy, unpublished), and some information about the breeding habits of *Pteropus giganteus giganteus* (Moghe 1952), *Cynopterus sphinx gangeticus* (Moghe 1956), and *Hipposideros bicolor pallidus* (unpublished personal observations) are known. By way of background the known information about the breeding habits of these animals is summarized here for ready reference. All the species, except *Taphozous*, breed only once in the year. In *Pteropus*, *Cynopterus* and *Megaderma* copulation occurs some time in early October (the season of copulation in *Cynopterus* may be spread out to about 4 weeks). In *Rhinopoma*, *Hipposideros* and *Scotophilus* copulation takes place in the month of March. *Taphozous* does not have a restricted breeding season, but breeds all the year round. In all these animals copulation is quickly followed by fertilization and pregnancy. In *Taphozous* there is a quick succession of pregnancies. In all the species, except in *Scotophilus,* only one side of the female genitalia is functional during any breeding cycle, a single ovum is released each time and a single embryo is borne in the respective uterine cornu. In *Scotophilus* both the ovaries ovulate during each breeding cycle, and an embryo is carried in each of the uterine cornua.

The significant and specific characteristics of the ovary become established only in the mature animal, and especially during the height of the breeding season. Hence the following descriptions mostly refer to the mature active ovary, and references to the quiescent ovary are made only where relevant. The phrase 'quiescent ovary' is used here to denote the ovary of the adult animal in the non-breeding season.

Size and gross appearance of the ovary

The normal size of the mature ovary in the various species of bats studied here is given below:—

> *Pteropus :* 3·0 to 4·2 × 2·0 to 2·8 mm.
> *Cynopterus :* 1·8 to 2·6 × 0·9 to 1·2 mm.
> *Rhinopoma :* 1·2 to 1·8 × 0·5 to 0·9 mm.

Taphozous : 1·5 to 2·1 × 0·6 to 1·0 mm.
Megaderma : 1·0 to 1·4 × 0·4 to 0·8 mm.
Hipposideros : 0·8 to 1·2 × 0·5 to 0·8 mm.
Scotophilus : 1·6 to 2·0 × 0·5 to 0·8 mm.

Although no direct correlation is noted between the size of the ovary and the size of the body of the bat possessing it, nevertheless it is interesting to note that the ovaries of these bats are relatively small as compared to the ovaries of other mammals having approximately the same body size, especially the polytokous ones. The small size of the ovaries in the bats may have some relation to the fact that, in most of the bats, a single young is born at a time, and even if two young ones are born as in *Scotophilus*, there is never more than one embryo in each uterine cornu. Since the maintenance of pregnancy is in some measure hormonally controlled by the ovary it is reasonable to assume that a single corpus luteum may be able to sustain the embryo in the uterus. It is further interesting to note that there is a direct connective tissue bridge between the ovary and the respective uterine cornu thus making possible the direct transport of progesterone from the ovary to the respective uterine cornu. This extraordinary utero-ovarian relationship has been reported in *Pteropus* (Marshall 1953), *Cynopterus* and *Taphozous* (Gopalakrishna and Murthy 1960). This special feature of the reproductive organs of the bats may also be, in some way, related to the small size of the ovary in these animals, since the small quantity of progesterone that is released from the ovary may be directly transported to the target organ—the associated uterine cornu. It is further significant that, in all the bats studied here, the corpus luteum was confined to the limits of the ovary and never projected beyond the surface, as reported in some rhinolophid bats (Matthews 1937). This fact further suggests that the structure of the ovary is essentially built on a mono-ovulatory plan.

Histological structure of the ovary

The histology of the ovary of the adult monotokous bats can be studied under four heads each having characteristic features: (i) the quiescent ovary; (ii) ovary during the oestrous cycle; (iii) the ovulated ovary in the pregnant animal; and (iv) the ovary on the non-ovulated side in the pregnant animal. Amongst the species studied here the last category does not apply to *Scotophilus* since, in this species, both the ovaries ovulate at each cycle and, therefore, present a similar histological picture during pregnancy.

(i) *The quiescent ovary :—*

In general the picture of the ovary during the quiescent period is almost the same in all the species. In none of the animals is there a well developed tunica albuginea, and hence a definite demarcation between the cortical and

medullary parts of the ovary cannot be made out. Here and there the germinal epithelium shows the presence of epithelial nodules—the fore-runners of the oocytes. It is thus obvious that the germinal epithelium continues to produce oocytes during the quiescent period. A few developing follicles are present in the deeper parts of the ovary; some of these follicles develop up to the multilaminar stage. However, all these follicles undergo atresia sooner or later.

(ii) *Ovary during oestrous cycle :—*

As can be expected the structure of the ovary during this period exhibits marked histological differences from the quiescent ovary, and it is at this time that the individual variations characteristic of each species become appa-rent. The growth of the oocyte and the follicle follows the same general plan as in other mammals, that is, during earlier stages of the development of the follicle the oocyte and the follicle grow concomitantly, but after the oocyte reaches a certain size, its rate of growth is retarded whereas the follicle grows enormously accompanied by the formation and the enlargement of the antrum.

During the early part of the oestrous cycle the ovary has a large number of follicles at various stages of development and some undergoing atresia (fig. 1). Follicular atresia might occur at any stage of the development of the follicle, and most commonly at the multilaminar and early vesicular stages. Except in *Taphozous* the histological appearance of the ovaries of the two sides is the same in all the species up to oestrum. In *Taphozous*, which experiences quick succession of pregnancies in alternate uterine horns, the ovary of only one side exhibits the oestrous changes, since the ovary on the opposite side has a postpartum condition and has the remnants of the corpus luteum of the previous pregnancy (Gopalakrishna 1955).

The development of the uni- and bilaminar follicles has been described in *Scotophilus* (Gopalakrishna 1948) and *Taphozous* (Gopalakrishna 1955), and this does not vary markedly in the other species studied here. The fully formed unilaminar follicle has a single layer of theca folliculi consisting of slightly flattened cells. The follicle cells are cuboidal to columnar, and their nuclei usually occupy the bases of the cells. The distal regions of the follicle cells have a vacuolated appearance. The zona pellucida has formed round the ovum although it is still thin.

A special feature of the bilaminar and multilaminar follicles of *Mega-derma* needs to be mentioned here. As the follicle grows into the bilaminar stage, there appears a circlet of vacuole-like fluid-filled spaces round the zona pellucida (fig. 7). These spaces are separated from one another by delicate membranes. Although these spaces give the appearance of the diatal vacuoles of the follicle cells surrounding the ovum, a closer examination and their future fate indicate that they are outside the follicle cells, and that they are

the beginnings of the antrum (*vide infra*). It thus appears that the antrum develops very precociously in this species, in the bilaminar stage of the follicle. Although the exact mode of origin of these spaces cannot be analysed in the material now available, their location and comparison with the follicles of the other bats studied here suggest that these spaces might have arisen by exudation of fluid from the follicle cells surrounding the ovum at the unilaminar and bilaminar stages. These vacuoles disappear as the antrum enlarges.

At the multilaminar stage (fig. 6) the follicle consists of 5 to 6 layers of cells in all the species. No distinction between the theca interna and theca externa can be made out. The basement membrane of the follicle is quite distinct. The fully formed multilaminar follicle is spherical in most species, but in *Cynopterus*, *Rhinopoma* and *Taphozous* there seems to be an overgrowth of the follicle on one side, so that it has a pear-shaped appearance with the ovum near the broader side of the follicle (fig. 3).

The mode of formation and the structure of the Graafian follicle present certain individual variations in the different species. In *Pteropus* and *Scotophilus* the formation of the antrum is initiated by the appearance of several small intercellular spaces within the follicle. The follicle cells adjacent to these intercellular spaces hypertrophy and contain fluid-filled vacuoles. Apparently the fluid in the vacuoles inside these cells exude and accumulate in the intercellular spaces resulting in the progressive enlargement of these spaces. The several antral spaces coalesce to form a large antral cavity in the fully formed Graafian follicle (fig. 10).

In *Megaderma* mention has already been made of the occurrence of vacuoles round the oocyte at the bilaminar stage of the follicle. The antrum makes its first appearance between the zona round the oocyte and the follicle cells by the coalescence of some of these vacuoles round the zona. Hence, in this region, the zona of the oocyte is directly bathed in the antral fluid. However, the zona soon becomes separated from the antral space by a single layer of cumulus cells (fig. 8).

Mention has already been made about the overgrowth of the follicle on one side in *Cynopterus*, *Rhinopoma* and *Taphozous* at the multilaminar stage of the follicle. The antrum first makes its appearance as one or more small spaces in the part of the follicle where the overgrowth has taken place, that is in the narrower part of the follicle (fig. 5). The growth of the antrum thus pushes the oocyte further towards the broader side of the follicle.

The structure of the fully formed Graafian follicle has been described in *Scotophilus* and *Taphozous* (Gopalakrishna 1948 and 1955). Apart from size differences, the Graafian follicle in *Pteropus* and *Cynopterus* presents similar histological picture to that of *Scotophilus*. Even at this stage no distinction can be made out between the theca externa and theca interna. The entire
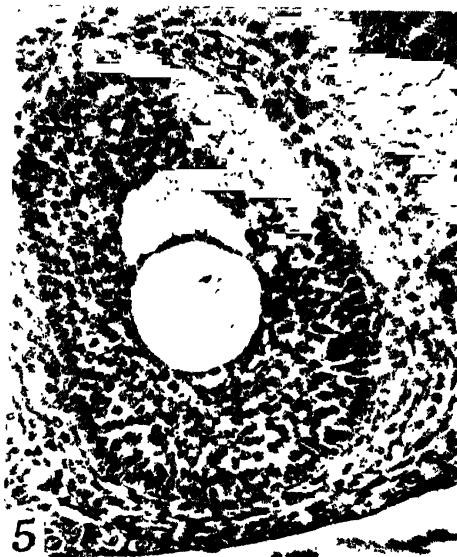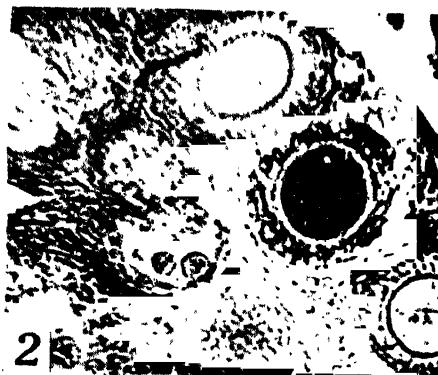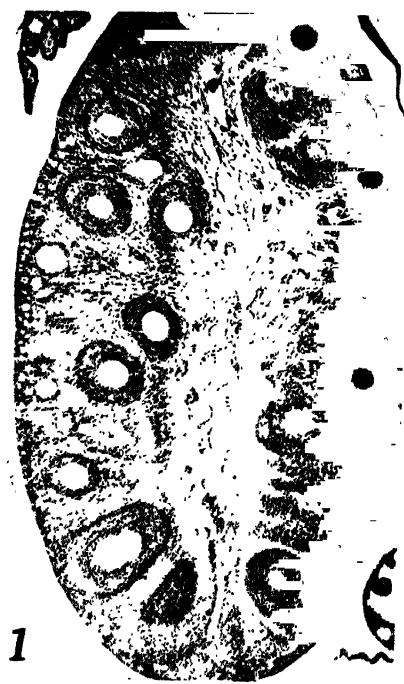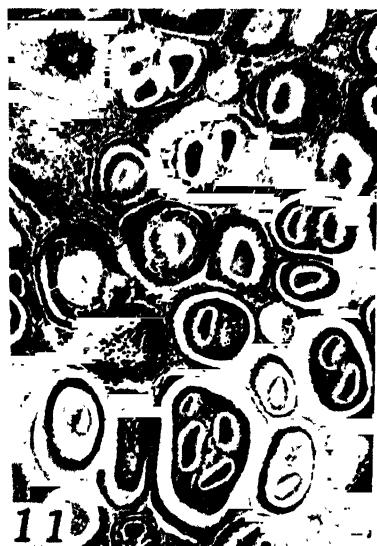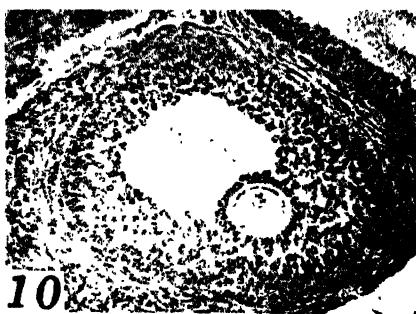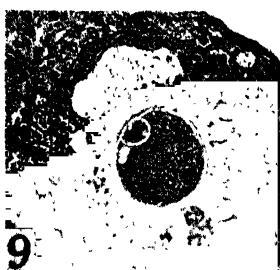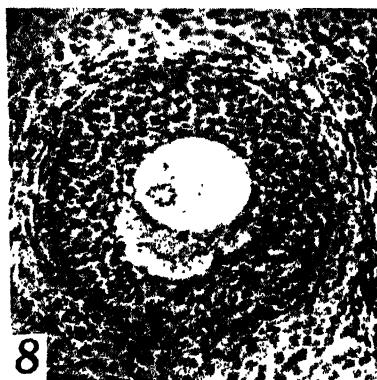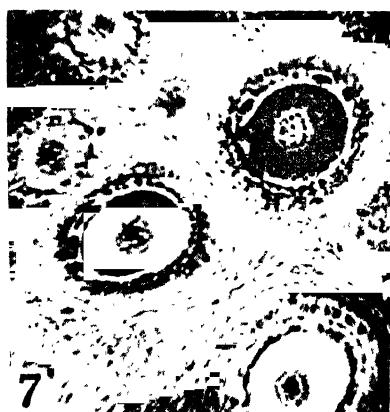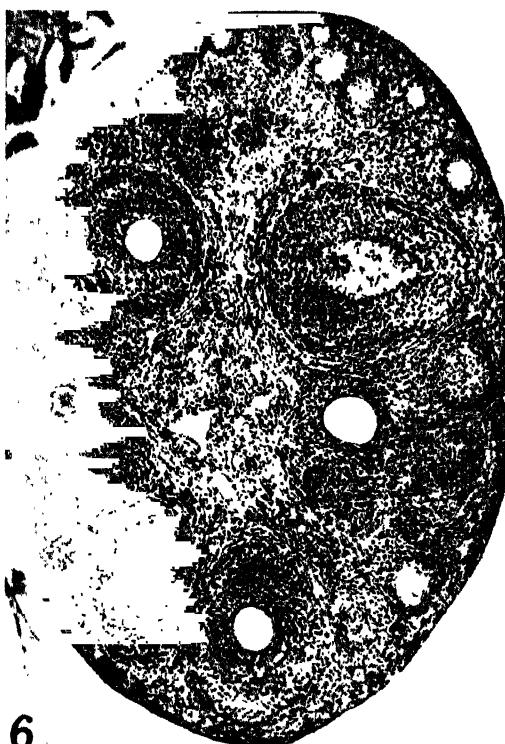
theca is made up of several layers of flat cells which give a fibrous appearance. The granulosa consist of 4 to 5 layers of closely packed small cells. The cumulus, the circlet of cells round the ovum, consists of 2 to 3 layers of similar small cells, and this is attached to the granulosa layer at one pole of the follicle by a narrow neck of cells. The rest of the follicle is occupied by a large antrum.

In *Rhinopoma, Hipposideros* and *Megaderma* the cumulus is made up of a single layer of small cells, and the ovum is broadly attached to the granulosa. The appearance is as if the oocyte is embedded in the granulosa at one pole of the follicle.

The ovary of *Megaderma* presents certain peculiarities which have not been described in any other bat, so far. During the early phases of the oestrous cycle, especially during pro-oestrum, a large number of bi-, tri-, and multi-ovular follicles are developed in addition to normal mono-ovular ones (fig. 11). The maximum number of ova recorded in a multi-ovular follicle was 6. It is interesting to note that the multi-ovular condition is not present during the earlier stages of the development of the follicle, that is during the uni- or bilaminar stages, but is seen only in more advanced follicles such as the multi-laminar and vesicular follicles. However, clusters of early follicles are often seen in the peripheral regions of the ovary. Theoretically, one could think of two methods of the formation of the multi-ovular follicle—either by fusion of two or more mono-ovular follicles or by an abnormal division of the oocyte within the mono-ovular follicle. In case the several ova within the multi-ovular follicle become functional, the genetic consequences of the two methods of development of the multi-ovular follicles would obviously be different. In *Megaderma* it is unlikely that the multi-ovular condition is accomplished by fusion of the adjacent follicles. On the other hand, this may be due to the division of the oocyte within the developing follicle. This conclusion is supported by the following observations. Although several specimens were examined, there was not a single instance where a bi-ovular follicle was noticed during the earlier stages of the development of the follicle, at least up to the multilaminar stage. If fusion of follicles takes place, then at least a few multi-ovular follicles should have been found in the uni- and bilaminar stages, since, after the multilaminar stage is reached, the theca folliculi becomes quite thick and acts as an effective barrier against fusion of the adjacent follicles. Secondly, division stages were noticed in the oocytes of some of the bilaminar follicles (fig. 2). These could not have been meiotic division stages since the first maturation spindle is not laid until after the follicle has reached the vesicular stage. Thirdly, the oocytes within the multi-ovular follicles are considerably smaller than the oocytes within the normal mono-ovular follicles, thus suggesting that the ooplasmic material of the oocyte might have been distributed among the daughter

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oocytes within the multi-ovular follicle. The physiological significance of the formation of so many multi-ovular follicles is not clear. All these abnormal follicles, however, undergo atresia during oestrum.

(iii) *The ovulated ovary during pregnancy :—*

The outstanding feature of the ovary on the pregnant side is the presence of the corpus luteum which lies within the confines of the ovary in all the species studied here. The growth of the corpus luteum is rapid, and, when fully developed, it occupies almost the entire ovary except for a small peripheral area. In the peripheral part of the ovary a few follicles continue to develop up to uni- or bilaminar stage, but all these follicles undergo atresia during pregnancy. In *Scotophilus*, in addition to the luteinization associated with the cells of the ruptured follicle, the cells of the theca interna and theca externa of the adjacent follicles also hypertrophy and exhibit characteristic changes stimulating the cells of the corpus luteum (fig. 4). This peculiar phenomenon seems to occur as a chain induction reaction since progressively the thecal cells of follicles lying quite remote to the ovulated follicle also undergo hypertrophy, and change so as to appear like luteal cells. By mid-pregnancy when the corpus luteum has developed to its maximum size, the rest of the ovarian medulla is occupied by masses of luteal-like cells derived from the adjacent follicles in which the oocytes have undergone degeneration. Very often the main corpus luteum can be distinguished from the accessory masses of luteal tissue by presence of tissue partitions.

(iv) *The non-ovulated ovary in the pregnant animal :—*

Mention has already been made of the fact that in all the species, except *Scotophilus*, only one side of the genitalia is functional during any breeding cycle. A single ovum is released from one of the ovaries, and hence the corpus luteum is present in only one ovary. Hence, in these species, the two ovaries present different histological pictures during pregnancy. Amongst these species, in *Taphozous*, since pregnancies follow in quick succession in alternate uterine horns, the remnants of the corpus luteum of the previous pregnancy persist for some time in the non-ovulated ovary during pregnancy. In the other species the non-ovulated ovary presents a typical anoestrous condition throughout pregnancy. In *Scotophilus*, since ovulation takes place in both the ovaries, the two ovaries present a similar picture, each with a corpus luteum.

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EXPLANATION OF FIGURES (PLATES III AND IV).

FIG. 1. Section of the ovary of *Taphozous longimanus* in early pro-oestrum. The ovary has follicles at various stages of development. A few follicles are undergoing atresia. Most of the follicles which are located in the deeper part of the ovary are multi-laminar. One of them (7 o'clock) shows antral cavity. × 60.

FIG. 2. Section of the ovary of *Megaderma lyra lyra*. The nucleus of one of the ova (3 o'clock) is undergoing division. A multi-nucleate condition is seen in the ovum in the adjacent follicle. This condition is the forerunner for undergoing atresia. × 160.

FIG. 3. Part of the ovary of *Taphozous longimanus* showing two well formed multilaminar follicles. Note the pear-shaped nature of the follicle. × 70.

FIG. 4. Two atretic follicles from the ovary of a pregnant specimen of *Scotophilus wroughtoni*. Note the hypertrophied cells of the theca simulating the cells of the corpus luteum. × 450.

FIG. 5. Pear-shaped follicle of *Taphozous longimanus* showing the formation of the antrum. Note a single layer of cumulus cells separating the zona of the ovum from the antral space. × 300.

FIG. 6. Section of the ovary of *Cynopterus sphinx gangeticus* (late pro-oestrum) showing several multilaminar follicles surrounded by several layers of theca cells. × 80.

FIG. 7. Bilaminar follicles of *Megaderma lyra lyra*. Note the occurrence of vacuoles round the zona. × 160.

FIG. 8. Vesicular follicle of *Megaderma lyra lyra*. Note a single layer of flat cells between the antrum and the zona. × 260.

FIG. 9. Early vesicular follicle of *Scotophilus wroughtoni*. The antrum is forming as several spaces. × 260.

FIG. 10. Fully formed Graafian follicle of *Scotophilus wroughtoni*. × 120.

FIG. 11. Section of the ovary of *Megaderma lyra lyra* showing several multi-ovular follicles. × 100.

NEW RECORD AND DESCRIPTIONS OF NEW SPECIES OF PARASITES OF SAN JOSE SCALE

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ABSTRACT

The author was named by the Indian Council of Agricultural Research in 1951 to make a survey of the Kashmir valley to find out the natural enemies of San Jose Scale (*Quadraspidiotus perniciosus* Comstock), a serious pest of apple and other deciduous trees, with a view to their eventual utilization, if possible, for the biological control of the pest. During the course of this survey the author recorded eight species of chalcidoid parasites and two coccinellid predators. In this paper the descriptions of *Marietta indica, Azotus kashmirensis, Ageniaspis indicus, Polynema anantanagana* and *Lymaenon pahlgamensis*, all new to science, have been given. *Casca chinensis* Howard has been recorded for the first time from the Indian Sub-continent.

INTRODUCTION

San Jose Scale, *Quadraspidiotus perniciosus* Comst. (Pl. V), is a destructive pest of deciduous fruit trees like apple, pear, peach, apricot, plum, cherry, etc., and is of world-wide distribution. Kashmir is well known for the excellence of the fruits that are grown in her fair valley. To some extent the prosperity of both the rural and urban population of Kashmir is dependent upon her horticultural industry. More than 500,000 maunds of all kinds of deciduous fruits are annually exported from the State. San Jose Scale seems to have entered Kashmir valley about five decades ago probably through defective quarantine regulations. Since then, the pest has, by its staggering rate of reproduction, spread over almost the entire Kashmir valley. Its host index has also increased and, besides the fruit trees, the pest has been observed to attack poplars, willows, maples, elms and even wild roses. It has even been observed to infest seasonal vegetable crops like radish, turnip, spinach, etc. The polyphagous habit of the pest renders the effective control of the pest a baffling problem to the economic entomologist. In 1939, the then Imperial Council of Agricultural Research initiated a scheme known as '*The San Jose Scale and the woolly aphis research scheme in Kashmir*'. The scheme ran for about seven years and as a result of repeated laboratory experiments and orchard practices it was found that spraying of fruit trees just after autumn with diesel oil emulsion and fish oil rosin soap was the most effective method of keeping the scale under check. During this period of seven years three unidentified parasites and eight predators, parasitizing or predating on San Jose Scale, were also recorded.

Experience, however, has shown that the insecticidal method of treating orchards year after year is not an economic proposition. There were other difficulties as well encountered in the control of the pest in the Kashmir valley. Even after the pest was effectively controlled in the orchard by means of insecticides, it was soon found that the pest re-infested the orchards from poplars and willows grown in the forest areas for afforestation in the highways as avenue trees and by the lake side for beautifying the landscape. It is almost impossible that the chemical method of control could be extended to all these areas and the cost of production kept within reasonable limits. So the biological method of control by means of the natural enemies of the pest suggested itself as one of the logical methods of control of the pest. With this end in view the author was deputed by the Indian Council of Agricultural Research in August 1951 to visit the Kashmir valley for a survey of the natural enemies of the pest in the orchards and plantations of the Kashmir valley.

The various places that the author visited and surveyed and the dates of his visit to these places are given in the map (Fig. 13). As a result of this survey eight species of parasites belonging to the super family Chalcidoidea were recorded, of which five were new to science and one recorded for the first time from the Indian Union. The remaining two species belonging to the genus *Aphelinus* are still under study.

Till now 32 species of Chalcidoid parasites belonging to the families Encyrtidae, Eulophidae, Aphelinidae and Mymaridae have been recorded from the fruit-growing regions of the world. The following six species of which five are new to science are described below. It may be mentioned that the genera, *Ageniaspis* Dahlb., *Casca* Howard, *Polynema* Haliday and *Lymaenon* Haliday, have been reared for the first time on this pest. Some notes have been added to the species that has been mentioned earlier as recorded for the first time from the Indian Union.

Family : APHELINIDAE

Marietta indica, new species (Figs. 1 and 2)

Female: Length 0·54 mm. Dark orange in colour. Head as wide as thorax; vertex smooth. Eyes dark brown; lateral ocellar space subequal to ocellocular space. Antennae brownish; scape pale yellow, ring joints dark brown, first and second funicular segments brownish, third funicular segment pale yellow, setae pale yellow.

Thorax—Dark orange; mesoscutellum smooth, scutellum smooth in front but finely striated behind. Forewings with reticulate pattern of brown colour; proximad of hairless line against origin of stigmal vein. Five small coarser spines on the marginal vein, spines pale yellow. Venation light

brown. Midway between the apex of venation and apex of wing a small area is covered by two rounded clear spaces. They are bounded by pale brown ciliation forming a complete figure of 8. A clear space bounded by pale brown cilia against the lower margin of the wing below the figure of 8. Hind wings relatively much narrower. Legs pale yellow, conspicuously banded with dark brown stripes; the first two bands on the hind tibia confluent with each other. Tibia with two encircling dark bands. Tarsal segments 1, 4 and 5 in all legs dark brown; 2 and 3 pale yellow.

Abdomen—Orange. Ovipositor exserted.

Male—Same as female. Slightly smaller in size.

Holotype—One female mounted in Canada balsam, labelled 'parasitic on San Jose Scale' collected: E. S. Narayanan, 28th August, 1951. Deposited in the National Pusa Collection, Indian Agricultural Research Institute, New Delhi.

Allotype—One male mounted on a slide in Canada balsam.

Locality—Kashmir.

Host—*Quadraspidiotus perniciosus* Comstock.

Azotus kashmirensis, new species (Figs. 3 and 4)

Female—Length 0·85 mm. Body brown. Eyes pinkish red, legs concolours with body. Anterior half of pedicel, second and fourth funicular joints, parts of femora and tibia and tarsi pale white. Ovipositor sheath yellowish brown.

Head transverse, slightly narrower than thorax. Eyes large but not covering the most part of the vertex. Antennae 7 jointed; scapula including the radicle at least three times longer than the pedicel, pedicel much longer than wide; funicular joint one is as long as two and third is smaller than two and four; club distinctly single jointed, the suture between the two joints totally obliterated; at least $2\frac{1}{2}$ times longer than the fourth funicular joint. Forewings nearly three times as long as broad, submarginal vein slightly longer than the marginal with a stout solitary bristle near the junction with the latter, stigmal vein short and stumpy with a prominent knob, marginal cilia longest near the anal angle, forewings with three distinct infuscated transverse bands; infuscated area beset with strong black short bristles; a group of strong black bristles more prominent than the transverse bristles just below the stigmal vein (15 bristles). Middle tibial spur shorter than the first tarsal segment. Abdomen broader than thorax. Ovipositor exserted, the length being less than half of abdomen.

Male—Shorter than female, slightly lighter in colour. The forewings are hyaline. But for these major differences the male is almost similar to female.

Azotus kashmirensis, new species, differs from the only other known Indian species *Azotus delhiensis* Lal by the following characters: The ratio of the

length of the antennal segments, the fuscas transverse bands of forewings and the length of the mid tibial spur.

Described from two females and one male.

Holo and allotypes on slides.

Host—Quadraspidiotus perniciosus Comstock.

Locality—Kashmir.

Collected by E. S. Narayanan, 1951.

Casca chinensis Howard (Fig. 5)

I refer to this species as *Casca chinensis* Howard. This is the first record of the genus and species from the Indian Sub-continent.

Host—Quadraspidiotus perniciosus Comstock.

. *Type locality*—Kashmir.

Collected by E. S. Narayanan, 1951.

Mounted on a slide.

Aphelinus species (Figs. 6 and 7)

This is a small deep yellow species more abundant than others. This species considerably differs from the introduced *Aphelinus mali* Hld. This species is distributed throughout the Kashmir valley.

Host—Quadraspidiotus perniciosus Comstock.

Type locality—Kashmir.

Collected by E. S. Narayanan, 1951.

Mounted on a slide.

Aphelinus species (Fig. 8)

This species which is dark brown and nearly twice the size of the yellow species is also found in large numbers.

Host—Quadraspidiotus perniciosus Comstock.

Type locality—Kashmir.

Collected by E. S. Narayanan, 1951.

Mounted on a slide.

Family : ENCYRTIDAE

Ageniaspis indicus, new species (Figs. 9 and 10)

Female—Length 0·99 mm. Body colour including head dark brown, eyes brick red, legs yellow with dark brown patches.

Head as wide as thorax, lateral ocelli separated from the eye border by a distance less than their diameter; eyes very finely pubescent, seen only under high magnifications. Scape and pedicel brown, flagellum and club pale yellow.

Scape more than twice as long as broad, pedicel longer than broad; first funicular joint the smallest. 1–4 broader than long, 5th longer than broad, 6th relatively thicker, club nearly three times the length of the pedicel, almost equivalent to the preceding four segments, very much thicker than the funicle, terminal segment pointed.

Thorax dark brown. Axillae distinctly separated. Propodeum almost yellowish, smooth, unsculptured. Forewings thrice as long as broad; post marginal is slightly longer than marginal, slightly shorter than the stigmal.

Gaster slightly shorter than the thorax. Paratergites prominent. Ovipositor exserted nearly half the length of the abdomen.

Male—Essentially similar to female except the antennae which are hairy, the funicles are of almost equal length.

Holo and Allotype on slide.
Host—*Quadraspidiotus perniciosus* Comstock.
Locality—Kashmir.
Collected by E. S. Narayanan, 1951.

Ageniaspis indicus comes close to *A. pyrillae* Mani but differs from it in the following: the coloration, measurements of the components of the antennae, wing venation and the exserted ovipositor.

Family : MYMARIDAE

Polynema anantanagana, new species (Fig. 11)

Female—1·1 mm. long.

Head and abdomen brown, thorax dark brown. Antenna except scape and pedicel, legs except hind femur are dark brown to brown. Tarsi, scape of antenna and pedicel light yellow. Eyes deep red.

Head a little wider than long, vertex almost smooth with a row of three stiff bristles on either side of the median ocellus. Eyes almost of the length of the genal spaces. Ocelli arranged in an obtuse-angled triangle; the lateral ocelli away from the eye margin by about its own diameter. Antenna long and slender, measuring to about 0·7 mm. in length; very slightly pubescent; radicle about 1/8th of the scape. Scape longer than broad, as broad as pedicel. Pedicel almost roundish, ½ of the length of scape. Funicles 1 to 6 slender, longer than broad, club nearly three times longer than its own breadth. The relative lengths of its segments from scape onwards as below:

$$8, 4, 3·5, 6, 4·5, 4, 3, 4, 12·5.$$

Club with 4 sensillae; the last funicle with only one. Mandibles distinctly 3 dentated, the teeth being equal though the inner tooth is slightly shorter.

Thorax as wide as head, narrow anteriorly, broader posteriorly. Abdomen with the petiole is longer than the thorax. Thorax almost smooth and

shining, without any hairs or bristles. Mesoscutum much longer than scutellum.

Petiole longer than the hind coxa. Abdomen conic ovate, ovipositor very short but evidently exserted.

Forewings hyaline, narrow and spoon-shaped; the currat slightly more pronounced on cephalic than on caudal margin. Discal cilia uniformly spread. Marginal fringe long, the longest of these being nearly half of the width of the forewing. Forewing length three times that of the greatest breadth.

Legs long and slender. Hind tarsus nearly equal to its tibia. Tibia longer than the combined lengths of femur and trochanter; meta-tarsus shorter than the rest of the tarsi combined.

Male—Not known.

Described from a single specimen mounted on slide.

Collected by E. S. Narayanan, 1951.

Locality—Anantanag, Kashmir.

Host—San Jose Scale, *Quadraspidiotus perniciosus* Comstock.

Lymaenon pahlgamensis, new species (Fig. 12)

Female—Length 0·91 mm. (including ovipositor).

Colour—Eyes black, ocelli dark brown, body yellow, legs concoloured with the body.

.   Head slightly longer than broad. Vertex flat and prominent like other species of the genus. Eyes black, elongate. Genal space slightly more than half the length of eyes. Ocelli arranged in a low arc. Interocular space less than three times that of interocellar and nearly six times that of ocellocular. Antennae yellow, situated slightly below the middle of the face; the relative lengths of the antennal segments from scape to club are as follows:

12, 4·5, 2, 2, 2, 3, 3·5, 4, 4, 3, 11·5.

Club solid, about three and one third its own breadth, with six linear sensillae. F_5, F_7 and F 58 with one sensilla each.

Thorax smooth, parapsidal furrows poorly defined. Upper half of the scutellum a shade darker than the rest which is yellow. Scutum equal to scutellum in length; metathorax very narrow, propodeum with a round spiracular sulci, a short bristle just below the sulci on each side.

Abdomen conic ovate; smooth, shining, upper half of the dorsum yellow, lower half brown. A row of bristles present on each of the tergites, the first row with only two bristles. Ovipositor slightly exserted.

Legs slender; coxae, femur and tibia yellow though tibia and tarsi are a shade darker. Hind tibia longer than the combined length of the femur and trochanter.

Wings—Forewings nearly four times as long as broad. Submarginal vein much longer than the marginal. Marginal cilia comparatively short, the longest being less than half the breadth of the wings.

Holotype- -One female on slide.

Host—*Quadraspidiotus perniciosus* Comstock.

Locality—Kashmir.

Collected by E. S. Narayanan, 1951.
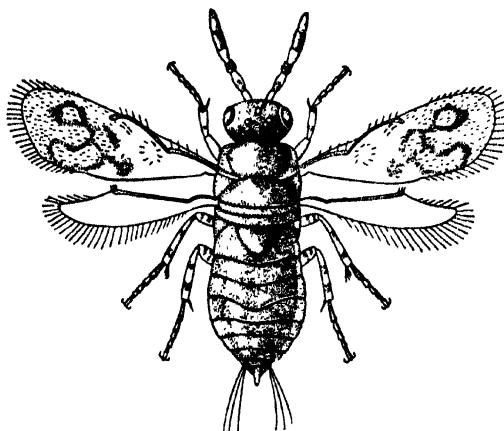
FIG. 1.   *Marietta indica*, new species, female.
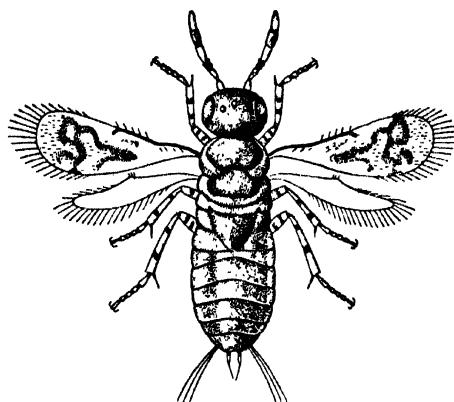
FIG. 2.   *Marietta indica*, new species, male.
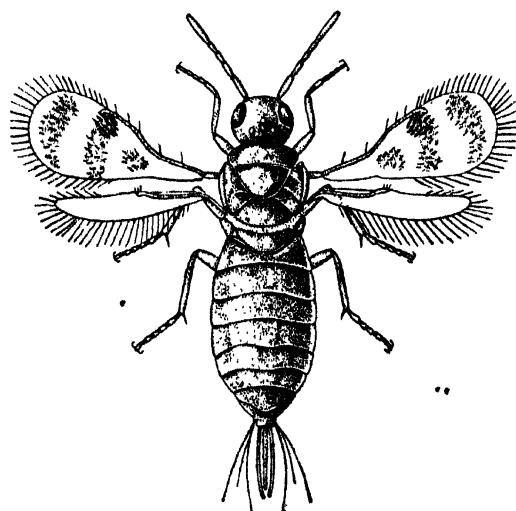
FIG. 3.   *Azotus kashmirensis*, new species, female.
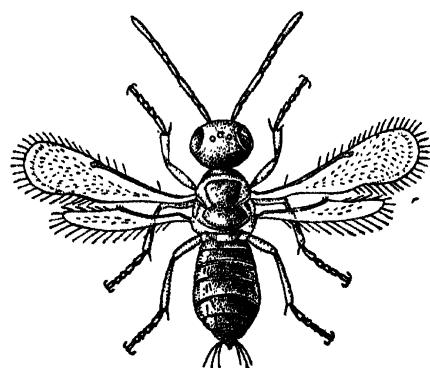
FIG. 4.   *Azotus kashmirensis*, new species, male.
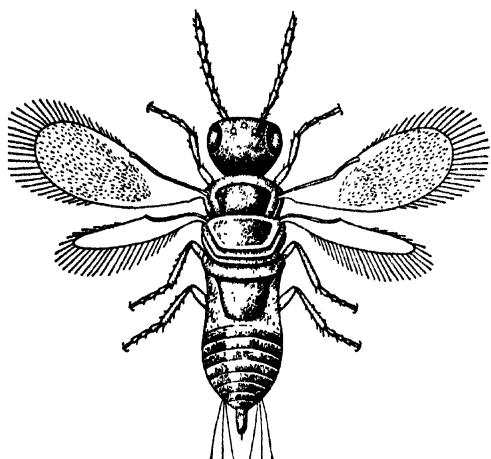
FIG. 5. *Casca chinensis* Howard.
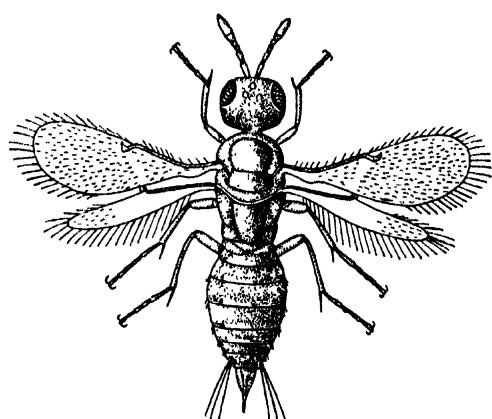
FIG. 6. *Aphelinus* species (yellow), female
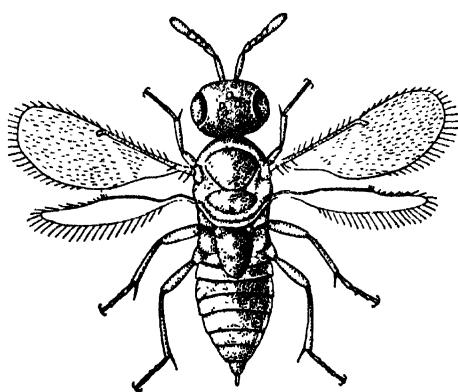
FIG. 7. *Aphelinus* species (yellow), male.
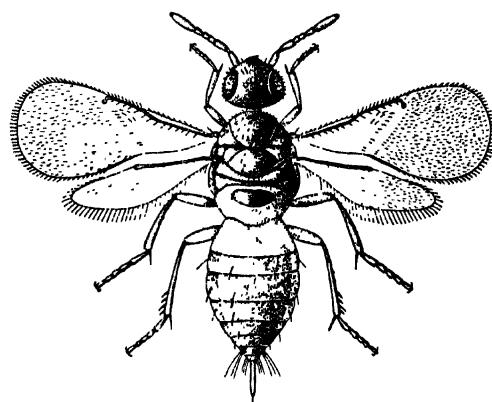
FIG. 8. *Aphelinus* species (brown), female.
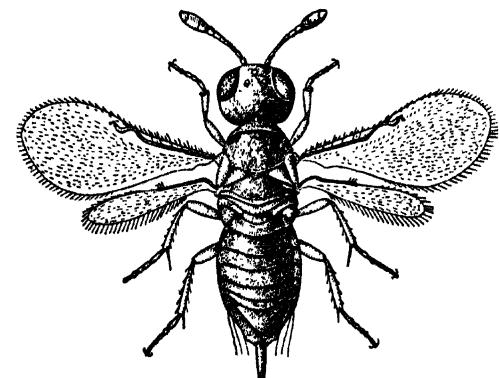
FIG. 9. *Ageniaspis indicus*, new species, female.
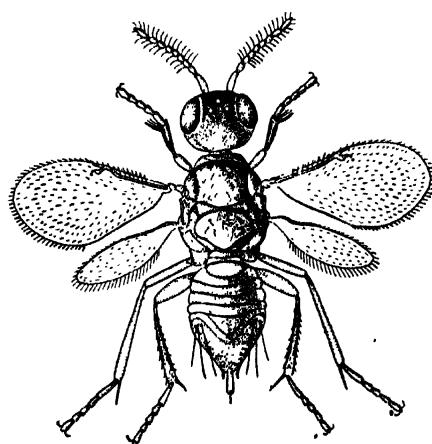
FIG. 10. *Ageniaspis indicus*, new species, male.
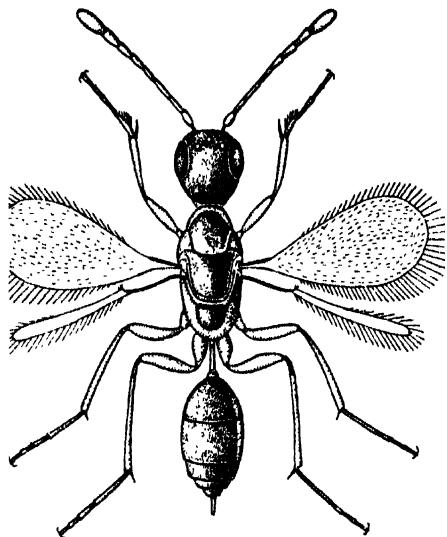
FIG. 11.   *Polynema anantanagana*, new species.

FIG. 12.   *Lymaenon pahlgamensis*, new species.

1

3

5

4

2

GILGIT

MUZAFFARABAD

LADAK

PUNCH

RIASI

UDHAMPUR

• ZACHALDARA
  28 8 51
• WATARIAN
  28-8-51
• WATALAB
  24 8 51
• WARIPORA

• WODORA
  28 8 51

• THAWAG AN SING
  GARDEN
  28 51

• SUNDRI NURSERY
  28 51
• NOAPURA
• DOABUA
  28 51
• SINGPURA GARDEN
• BARAMULLA 29 8 51
  29 8 51
• SOPOR
  28 51
• HANJ SING
  28 51
• SANGRAMA
• AMARGARH
  21 8 51

• ASHAM
  30 51

• RAIPUR

• GANDARBAL

• DRAPAHAMA
  2 9 51

• SAMINAL
  30 51
• MAR KRANDEL
  30 51
• ZAZUNA
  30 8 51

• FATCHPUR
  30 51
• HARWAN 2 9 51
  31 51
• SHAL AMAR
  31 51

• KHREW
  31 8 51

• PATTAN
  30 51
• KHIMCHAND
  31 51
• NARBAL
  7 9 51
• HAZATBAL
  31 8 51
• DAYAKISHAN
  GARDEN
• THE IDGA 21 11
• NANDPURA 31 51
• SRINAGAR
• LALMANDI
• HAWAL PURA
  1 9 51

• NISHAT
  31 51
• CHASMASHI
  2 9 51
• RAJBAGH
  1 9 51

• ZOWUR
  31 8 51
• KHNUMOH
  31 8 51

• TANGMARG
  7-9-51

• LALDAR
  GARDEN
  1 9 51

• KAKPORA
  1 9 51
• MARVAL
  8 9 51
• POHU
  8 9 51

• SHUPIYAN
• DEGAM
  8 9 51

• PANHPORA
  3 9 51
• PHALGAM
  3 9 51
• AISH MUKAM
  3 9 51

• KHUD WANI
  3-9 51

• ANANT NAGH
  3 9 51
• ACHABAL
  3 9 51
• HAKHOORA
  3 9 51

• KOKARNAGH
  3-9-51
• DAMHALL
  3 9 51
• HAIKHORA
  3 9 51

WAYAL •
3-9 51

• VERINAG
  4 9 51
• SUBAGING
  GARDEN
  4 9 51

MAP OF KASHMIR VALLEY SHOWING
PLACES VISITED BY THE AUTHOR
DURING AUGUST-SEPTEMBER 1951

FIG. 13.

BIOASSAY STUDIES OF THE CATFISH AND SKIPPER-FROG PITUITARY GLANDS AFTER TREATMENT WITH SOME GLYCOPROTEIN SOLVENTS

by L. S. RAMASWAMI, F.N.I., *and* A. B. LAKSHMAN,* *Animal Physiology Section, Department of Zoology, Central College, Bangalore*

ABSTRACT

Bioassay experiments have been conducted to study the extraction properties of 1·25 and 2·5 per cent trichloracetic acid, half-saturated ammonium sulfate and 50 per cent pyridine on the glycoprotein content of pituitary glands of the catfish (*Heteropneustes fossilis* (Bloch)) and of the skipper-frog (*Rana cyanophlyctis* Schn.). The pituitary glands were immersed in 1·25 and 2·5 per cent trichloracetic acid (TCA) for 6, 12 and 24 hr. duration; in 2·5 per cent TCA the pituitary glands of the skipper-frog were left for 36 hrs. also. Both the immersed gland and the TCA in which the glands were immersed were injected into test animals, maintaining suitable controls. Only glands immersed in half-saturated ammonium sulfate and 50 per cent pyridine were injected as these fluids are lethal to animals. It was found that catfish pituitary glands immersed in 6, 12 or 24 hrs. in 2·5 per cent TCA and the TCA fluid in which the glands were immersed brought about no ripening of eggs in the gravid catfish; both the pituitary glands and the 1·25 per cent TCA fluid in which they were immersed for 6 or 12 hrs. brought forth ripe eggs in catfish while the glands immersed for 24 hrs. in this concentration of the acid as also the fluid in which they were immersed failed to ripen the eggs of the catfish. In the skipper-frog the TCA (1·25 or 2·5 per cent) in which the pituitary glands were immersed over the different intervals of time gave negative results; no ripening of the eggs was seen. The pituitary glands immersed in 2·5 per cent TCA for 6 or 24 hrs. of a few skipper-frogs yielded very few eggs on injection while those immersed in 1·25 per cent TCA gave a large number of eggs in the skipper-frog; the ripening of a few eggs in the former is due to the residual luteinizing hormone in the treated pituitary gland and as it happened only in one or two specimens, the retention of the luteinizing hormone may be fortuitous. In the skipper-frog the pituitary gland-immersed fluid uniformly failed to bring about the ripening of eggs. This may mean that the TCA does not extract the glycoprotein hormones in the skipper-frog but denatures and renders them inactive in the gland itself. In this respect, the action of the extracting fluid is different in the catfish from that in the skipper-frog when pituitary glands are immersed in TCA. Half-saturated ammonium sulfate does not extract the glycoproteins from the pituitary glands and both catfish and skipper-frog yield ripe eggs on receiving it; 50 per cent pyridine completely extracts the glycoproteins and the pituitary glands fail to ripen the eggs of the catfish and of the skipper-frog.

INTRODUCTION

Pituitary cytology has been studied largely on mammalian material and there have been very few accounts of the same in lower vertebrates. Being familiar with the spawning reactions of catfish and frogs, it was our intention

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to study the pituitary cell types and their probable functions in order to correlate the activity of the pituitary gland with reproductive behaviour.   In this connexion, our digestion experiments (Ramaswami and Sundararaj 1958; Ramaswami and Lakshman 1960) have proved the existence of the luteinizing hormone in the pituitary glands of the catfish and of the skipper-frog.   The pituitary glands of the lower vertebrates, therefore, also elaborate a hormone similar to the mammalian luteinizing hormone and this alone can bring about spawning in the catfish and in the skipper-frog, and in the latter, an injection of a combination of trypsin or pepsin digested homogenate, which is assumed to contain the follicle-stimulating factor, and the luteinizing hormone-containing (ptyalin digested) homogenate did not bring about more spawning than when the luteinizing hormone-containing homogenate alone was used.   We have, however, not assayed to prove the presence of follicle-stimulating hormone in the catfish or in the skipper-frog.   Adams and Granger (1938) have shown that the anterior part of the pituitary gland of the frog can cause the growth of follicles in infantile mice indicating the presence of follicle-stimulating hormone.   Otsuka (1957a) has extracted follicle-stimulating and luteinizing hormones from salmon pituitary glands.

Taking advantage of the differential solubility of glycoproteins of the pituitary glands on immersion in certain fluids, Barrnett *et al.* (1956) used the treated glands not only for bioassay but also for cytochemical studies.   As a result of their studies on male albino rats, they were able to show that 2·5 per cent trichloracetic acid (TCA) extracted both the follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH) but the luteinizing hormone (LH) was precipitated, which, when assayed, brought about extraordinary decrease in ovarian, uterine and testicular weights as compared with results obtained with normal gland.   Enough gonadotrophin was present in the extracted gland to maintain testis weight and of the prostate above controls killed seven days after hypophysectomy.   50 per cent pyridine extracted all the glycoproteins from the pituitary glands and bioassay results were almost identical with those of controls killed seven days after pituitary ablation. Half-saturated ammonium sulfate solution ($\frac{1}{2}$ SAS) did not extract any of the glycoprotein hormones (except very little of FSH) and the bioassay showed a reduction in the weight of the recipient ovaries, uterus and seminal vesicles when compared with test animals receiving pituitary glands of normal animals.   This extracting fluid is less efficient in its capacity to extract gonadotrophins than sodium sulfate solution as disclosed by bioassay results. Histologically pituitary glands showed greater change in these three fluids than in TCA.   With regard to the results obtained with TCA fluid, Barrnett *et al.* (1956) came to the important conclusion that 'The findings in both bioassay and histochemical material clearly indicate that the procedure of extracting the pituitaries with 2·5 per cent TCA, refixing in sublimate-formol

and then staining by the PAS method provides a histochemical method for the determination of LH.'

In this paper, we propose to describe the results of our bioassay experiments on catfish and skipper-frog females after injecting them with homoplastic pituitary glands immersed in glycoprotein solvents. In a later communication, we propose to describe the cytodifferentiation of both the normal and the treated pituitary glands.

MATERIAL AND METHODS

A known number of pituitary glands was taken out of freshly brought eggbound catfish (*Heteropneustes fossilis* (Bloch)) females and of skipper-frog females (*Rana cyanophlyctis* Schn.) and separately immersed at room temperature for 24 hrs. in a known volume of each of the following fluids: (a) 1·25 and 2·5 per cent TCA, (b) half-saturated ammonium sulfate solution and (c) 50 per cent pyridine. A quantity of the immersed pituitary gland corresponding to the untreated gland which brought forth ripening of eggs in the control was injected into the test animals. Parenteral injections of a volume of TCA containing the extracted hormone corresponding to that in the immersed gland injected were given to eggbound catfish; in some cases, however, a greater quantity of the fluid was injected to see if a greater quantity of the extracted hormone would bring about ripening of eggs. Similarly each catfish of another set received a quarter or half or two of the immersed catfish pituitary glands in quarter ml. of distilled water. Some test animals also received a combination dose of quarter treated gland plus TCA fluid containing extracted gonadotrophins corresponding to a quarter pituitary gland to find out if any small amount of LH left behind in the gland would synergically be augmented by other pituitary hormones. As pyridine and ammonium sulphate are lethal to animals, only the glands immersed in ½ SAS and in 50 per cent pyridine were administered to eggbound catfish and gravid skipper-frog females exactly like the TCA-treated ones. With regard to the TCA fluid we have not only immersed the catfish and skipper-frog pituitary glands for 24 hrs. but also for 6 and 12 hrs. respectively to study if this made any difference in the extraction of the hormones. Further, we have also reduced the concentration of the TCA fluid from 2·5 to 1·25 per cent and studied the extraction after immersion for 6, 12 and 24 hr. periods. Controls for each set of experiments of the catfish and the skipper-frog were maintained; each control catfish received either a quarter, or half or two untreated pituitary glands according to the quantity of the gland injected to the experimentals; similarly each skipper-frog control received three or four or five untreated pituitary glands according to the number of glands injected into the test skipper-frogs. This enables us to get an idea that the quantity of pituitary glands injected

was sufficient to bring forth ovulation normally.   With regard to the skipper-frogs, treated glands were homogenized in half to one ml. of distilled water and injected; a quantity of the TCA fluid in which the glands were immersed corresponding to the quantity of treated pituitary glands was also injected into another set.   The reactions have been studied and tabulated.   Invariably more than five animals have been taken for each test.

OBSERVATIONS

We propose to describe the experiments on the two sets of animals separately :—

Pituitary glands of catfish immersed in 2·5 per cent TCA : It was noticed that gravid *Heteropneustes* did not yield ripe eggs on receiving either the TCA fluid (2·5 per cent) in which the homoplastic pituitary glands were immersed for 6, 12 or 24 hrs. (Table 1: Expts. 1–9).   Also the glands immersed in the TCA fluid for 6, 12 or 24 hrs. when injected did not bring about spawning in the test animals (Expts. 1–9) except, however, on one occasion (Expt. 5) when we were able to get 3 or 4 ripe eggs from a specimen receiving the 24-hr.-treated gland; this was probably due to the retention of a little LH in the gland.   In Expts. 2 and 9, a greater volume of the extracting TCA fluid was injected to find out if the apparently larger quantity of the extracted hormone would bring about ripening; this also did not give ripe eggs.   The combination of TCA-extracted gland and the TCA fluid in which the pituitary glands were immersed also did not cause ripening of eggs in the catfish and this experiment is not included in Table 1.

Pituitary glands of catfish immersed in 1·25 per cent TCA : Since the 2·5 per cent TCA-treated pituitary gland and the TCA fluid (in which the glands were immersed) did not cause spawning in *Heteropneustes*, we reduced the concentration of the TCA fluid from 2·5 per cent to 1·25 per cent to study its extraction properties.   It was noticed that when experiments were conducted immersing pituitary glands for 6 or 12 hrs. in 1·25 per cent TCA, the bioassay results were strikingly different.   Both TCA fluid and the extracted glands when injected separately brought forth spawning in the test animals in all cases (Table 1: Expts. 10, 11, 15 and 16).   Pituitary glands immersed in 1·25 per cent TCA for 24 hrs. or the gland-immersed fluid (Expts. 12–14, 17) brought about no ripening of eggs in the catfish.

Pituitary glands of catfish immersed in half-saturated ammonium sulfate and 50 per cent pyridine respectively : The results with the above two extracting fluids agree with those obtained for rat (Barrnett *et al.* 1956).   Ammonium sulfate solution does not extract the glycoproteins from the pituitary glands and when the immersed glands were injected, the test animals spawned yielding ripe eggs (Expts. 3, 4).   With regard to pyridine, the fluid extracted

3

TABLE 1

Action of glycoprotein solvents on the catfish pituitary gland

2·5 per cent trichloracetic acid

Expt. No.	Date	Control Weight range	Control Pit. gl. qty.	Control Eggs ripe + no −	Immersed gland Hrs. of immersion	Immersed gland Weight range	Immersed gland Pit. gl. qty.	Immersed gland Eggs ripe + no −	Fluid only Weight range	Fluid only Qty. of fluid	Fluid only Eggs ripe + no −
		gm.				gm.			gm.		
1	1959 Aug.	51	1/16 gl.	+	24	52–64	1/16 gl.	−	51	1 ml. = 1/16 gl.*	−
2	Sept.	67	1/16 gl.	+	24	56–98	1/16 gl.	−	51–121	1 ml. = ½ gl.	−
3	Oct.	63–112	¼ gl.	+	24	73–117	¼ gl.	−	69–112	½ ml. = ¼ gl.	−
4	Oct.	63–84	¼ gl.	+	24	46–96	¼ gl.	−	46–92	½ ml. = ¼ gl.	−
5	Oct.	63–89	¼ gl.	+	24	58–95	¼ gl.	+	55–86	½ ml. = ¼ gl.	−
6	Oct.	90–103	¼ gl.	+	24	61–104	¼ gl.	−	126–127	½ ml. = ¼ gl.	−
7	Nov.	80–102	¼ gl.	+	12	46–109	¼ gl.	−	58–106	½ ml. = ¼ gl.	−
8	Nov.	76–98	½ gl.	+	6	42–66	¼ gl.	−	50–100	½ ml. = ¼ gl.	−
9	Nov.	..	..	+	6	42–59	¼ gl.	−	45–58	½ ml. = ¼ gl.*	−

1·25 per cent trichloracetic acid

Expt. No.	Date	Control Weight range	Control Pit. gl. qty.	Control Eggs ripe + no −	Immersed gland Hrs. of immersion	Immersed gland Weight range	Immersed gland Pit. gl. qty.	Immersed gland Eggs ripe + no −	Fluid only Weight range	Fluid only Qty. of fluid	Fluid only Eggs ripe + no −
10	Dec.	87–104	1 gl.	+	6	88–121	1 gl.	+	82–127	½ ml. = 2 gl.*	+
11	Dec.	59–76	1 gl.	+	12	47–113	1 gl.	+	66–86	½ ml. = 2 gl.*	+
12	1960 Jan.	35–43	¼ gl.	−	24	78–103	1 gl.	−	58–62	1 ml. = 2 gl.*	−
13	Apr.	..	..		24	42–79	½ gl.	−	41–53	½ ml. = 1 gl.	−
14	Apr.	..	..	+	24	54–64	1 gl.	−	57–76	½ ml. = 1 gl.	−
15	Apr.	..	..	+	6	52–58	1 gl.	+	56–68	½ ml. = 1 gl.	+
16	Apr.	58–67	2 gl.		6	64–79	2 gl.	+	58–78	½ ml. = 2 gl.	+
17	Apr.	60–65	2 gl.		24	63–79	2 gl.	−	65–89	½ ml. = 2 gl.	−

* More quantity of the extracting fluid has been injected than the corresponding treated gland injected into another set.

TABLE 1—*contd.*

Action of glycoprotein solvents on the catfish pituitary gland

Expt. No.	Date	Half-saturated ammonium sulfate			50 per cent pyridine		
		Immersed gland			Immersed gland		
		Weight range gm.	Pit. gl. qty.	Eggs ripe + no −	Weight range gm.	Pit. gl. qty.	Eggs ripe + no −
1	Aug.	..	..		47-55	1/16 gl.	—
2	Sept.	114	1/16 gl.	died	..	..	
3	Oct.	55–104	¼ gl.	+	57–118	¼ gl.	—
4	Oct.	49–106	¼ gl.	+	46–108	¼ gl.	—

all the glycoproteins and when the extracted glands were injected, the test animals did not spawn (Expts. 1, 3, 4). It has already been pointed out that fluids used for extraction in these experiments are lethal and, therefore, they were not injected into test animals.

We are now going to describe the results of experiments conducted as above using the skipper-frog as the test animals.

Pituitary glands of the skipper-frog immersed in 2·5 per cent TCA : We have noticed that, in a few experiments, one or two skipper-frogs receiving pituitary glands immersed in 2·5 per cent TCA for 6 hrs. (Table 2: Expt. 7) or 24 hrs. (Expts. 4–6) yielded ripe eggs; the other skipper-frogs in the above experiments and also those receiving parenteral injections of pituitary glands immersed for 12 hrs. (Expts. 7, 8) or 24 hrs. (Expts. 3, 4–6, 8) in 2·5 per cent TCA yielded no ripe eggs. The TCA fluid (Expts. 1–8) in which the pituitary glands were immersed when injected always gave negative results, i.e. no ripe eggs.

Pituitary glands of the skipper-frog immersed in 1·25 per cent TCA : As in the catfish, we have also used 1·25 per cent TCA to study the degree of extraction. When the glands were injected after immersion in 1·25 per cent TCA for 6 or 24 hrs. (Expt. 10), the gravid skipper-frogs uniformly yielded ripe eggs; the TCA fluid in which the pituitary glands were immersed when injected did not yield any ripe eggs.

Pituitary glands of the skipper-frog immersed in half-saturated ammonium sulfate and 50 per cent pyridine : Pituitary glands of the skipper-frog immersed in ½ SAS when injected made the skipper-frog yield ripe eggs. Pituitary glands immersed in 50 per cent pyridine did not cause the skipper-frogs to yield ripe eggs on receiving them.

TABLE 2

Action of glycoprotein solvents on the skipper-frog pituitary gland

Expt. No.	Date	Control Weight range gm.	Control Pit. gl. qty.	Control Eggs ripe + no −	Immersed gland (2·5 per cent trichloracetic acid) Hrs. of immersion	Immersed Weight range gm.	Immersed Pit. gl. qty.	Immersed Eggs ripe + no −	Fluid only (2·5 per cent trichloracetic acid) Weight range gm.	Fluid Qty. of fluid	Fluid Eggs ripe + no −
	1959										
1	Aug.	34–40	4 gl.	+	24	34–41	4 gl.	+	36–41	1 ml. = 4 gl.	—
2	Sept.	38–44	4 gl.	+	..	..	3 gl.	.	..	1 ml. = 3 gl.	:
3	Sept.	42–44	3 gl.	±	24	41–47	4 gl.	—	44–49	1 ml. = 4 gl.	—
4	Sept.	34–49	4 gl.	+	24	36–38	4 gl.	±	36–40	1 ml. = 4 gl.	—
5	Oct.	31–40	4 gl.	+	24	33–44	4 gl.	±	34–46	½ ml. = 4 gl.	—
6	Dec.	32–42	5 gl.	+	24	34–41	5 gl.	±	36–38	1 ml. = 5 gl.	—
	1960										
7	Feb.	31–34	4 gl.	+	6	35–39	4 gl.	±	30–36	1 ml. = 4 gl.	—
					12	34–41	4 gl.	—	35–42	1 ml. = 4 gl.	—
					12	32–42	4 gl.	—	34–49	1 ml. = 4 gl.	:
8	Mar.	29–34	4 gl.	+	24	29–39	4 gl.	—		.	:
9	May	31–39	3 gl.	+	36	33–41	3 gl.	—		.	
						1·25 per cent trichloracetic acid					
10	Feb.	33–41	4 gl.	+	6	34–40	4 gl.	+	33–43	1 ml. = 4 gl.	—
					24	33–39	4 gl.	+	33–41	1 ml. = 4 gl.	—

TABLE 2—contd.

Action of glycoprotein solvents on the skipper-frog pituitary gland

Expt. No.	Date	Half-saturated ammonium sulfate			50 per cent pyridine		
		Immersed gland			Immersed gland		
		Weight range gm.	Pit. gl. qty.	Eggs ripe + no −	Weight range gm.	Pit. gl. qty.	Eggs ripe + no −
1	Aug.	..	..		..	..	
2	Sept.	..	..		36–45	4 gl.	
3	Sept.	46–48	3 gl.	+	44–48	3 gl.	
4	Sept.	35–46	4 gl.	+	38–42	4 gl.	
5	Oct.	34–43	4 gl.	+	32–42	4 gl.	
6	Dec.	32–41	5 gl.	±	31–40	5 gl.	

DISCUSSION

We have noticed that an injection of the catfish pituitary gland after immersion for 6 or 12 hrs. in 1·25 per cent TCA brought forth ripe eggs in eggbound catfish females; when the gland was immersed for 14 hrs. in 1·25 per cent or 2·5 per cent TCA, neither the gland nor the TCA fluid in which the pituitary glands were immersed brought forth ripe eggs in test animals receiving injections. If what Barrnett *et al.* (1956) have described for the TCA immersed rat pituitary gland also takes place in the lower vertebrate pituitary gland, the fluid would have extracted FSH and TSH leaving behind a major portion of LH in the gland. Our experiments with 2·5 per cent TCA indicate that neither the gland nor the extracting fluid has any LH factor in it as no ripening of eggs of catfish was brought about by them. Extraction experiments for 6 or 12 hrs. with 1·25 per cent TCA indicate that the fluid extracts partly the LH leaving behind a certain amount of the same gonado-trophin in the gland as both bring about spawning in the catfish. The spawning brought about in the catfish test animals (with the extracting TCA fluid or with the extracted pituitary gland as described above) may be exclu-sively due to LH as it has been found that ptyalin digested catfish pituitary gland homogenate containing LH factor alone brought about spawning in the catfish (Ramaswami and Sundararaj 1958); however, we do not rule out a certain amount of synergic action of the hormones in the extracted fluid. In the light of the above, if we examine the results obtained with the injections of pituitary glands immersed in 1·25 per cent TCA for 24 hrs. or in higher con-centration of TCA (2·5 per cent), the fluid probably not only extracts the glycoproteins but also denatures them as the test animals receiving it do not yield ripe eggs; the extracted pituitary gland also failed to ripen the eggs of

the test animals as the gonadotrophin was completely extracted and inactivated.

With regard to the skipper-frog, it has been found that the pituitary glands after extraction with 2·5 per cent TCA for 24 hrs. and in one case for 6 hrs. made one or two test animals yield ripe eggs. It is very likely that a small amount of LH was present in the pituitary gland after immersion in TCA fluid which was responsible for ripening a few eggs of the test animals indicated above. When the pituitary glands of the skipper-frog stay in the 2·5 per cent TCA for 36 hrs. the absence of LH in the pituitary gland is inferred as these do not cause even a few eggs to ripen in the skipper-frog. On the other hand, skipper-frog pituitary glands immersed in 1·25 per cent TCA for 6 or 24 hrs. caused the test animals to yield ripe eggs. Obviously the pituitary gland has not lost all its LH content. An amount sufficient to cause the ripening of eggs of the skipper-frog is present. The extracting TCA fluid whether of 1·25 or 2·5 per cent concentration does not contain any LH as the fluid when injected into gravid skipper-frog does not cause the ripening of eggs. The result obtained with the extracting fluid is very suggestive. While in the catfish the extracting fluid in which the pituitary gland was immersed for 6 or 12 hrs. brought forth ovulation in test animals on injection, in the skipper-frog the extracting fluid contains no LH as it does not bring about ripening of eggs in the gravid skipper-frog females. It leads us to infer that in the skipper-frog the TCA may not extract the glycoprotein but denatures it in the gland itself. If on the other hand, the glycoprotein is extracted by the TCA, then it is rendered inactive very quickly. In all these experiments we have assumed the ripening of eggs as an endpoint indicative of the presence of LH; it is likely that the gland after immersion may retain a small quantity of LH which may not be sufficient to cause ripening of eggs in the catfish or in the skipper-frog.

With regard to the other two solvents, viz. $\frac{1}{2}$ SAS and 50 per cent pyridine, the pituitary glands of both catfish and the skipper-frog do not lose their LH content after immersion in the former fluid as the respective test animals yield ripe eggs after receiving the immersed pituitary gland; Otsuka (1957b) also states that FSH and LH are not extracted by 0·5 or 0·8 saturated ammonium sulfate solution and that a 2 per cent sodium chloride solution extracts the FSH from frog pituitary glands. 50 per cent pyridine extracts all the glycoprotein hormones from the immersed glands and the catfish and skipper-frog test animals do not yield ripe eggs.

We are following up the bioassay studies by an examination of the histology of the pituitary glands with a view to identifying the LH secreting cells as described by Barrnett et al. (1956) for the rat. The cytology of the normal and the glycoprotein extracted pituitary glands of the catfish and of the skipper-frog using specific staining reactions for glycoproteins will be described later.

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PREVENTION OF CADMIUM INDUCED CHANGES IN THE GONADS OF RAT BY ZINC AND SELENIUM—A STUDY IN ANTAGONISM BETWEEN METALS IN THE BIOLOGICAL SYSTEM

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Abstract

The degenerative changes induced by Cd in the rat gonads are prevented by Zn and Se. It is possible that Cd is antagonized by the latter metals at two different sites.

It has been reported that Zn can prevent the degenerative changes in the testis of rats induced by $CdCl_2$ (Parizek 1957). Recently, Kar et al. (1959) have recorded analogous changes in the rat ovary after administration of $CdCl_2$. However, it is not known whether Zn can exert a salutary effect on the ovary of animals treated with $CdCl_2$. Further, antagonistic effect of metals other than Zn on Cd vis-à-vis prevention of gonadal degeneration is not on record.

The present paper is accordingly concerned with an attempt to investigate any antagonistic effect of Zn and Se on Cd with reference to destructive changes in the male and female gonads.

Experimental Procedure

Colony bred male (138–200 gm.) and female (40–70 gm.) albino rats of the Institute were used in this investigation. The details of grouping of the animals for different experimental purposes are indicated in Tables 1 and 2. The animals were maintained under uniform laboratory conditions throughout the period of study.

Cadmium chloride, $Zn (CH_3CO_2)_2$ and SeO_2 were administered by the subcutaneous route. In animals which received $CdCl_2$ and $Zn (CH_3CO_2)_2$ (or SeO_2) conjointly, the injections were given at different sites. A single injection of the metallic salts were given except in case of $Zn (CH_3CO_2)_2$ which in one set of experiment was administered at the rate of 8 m. mol/kg. (200 times the dose of $CdCl_2$). The total amount of 8 m. mol./kg. was split into 3 equal doses of 2·66 m. mol/kg. each and administered in the following manner: (1) 5 hours before injection of $CdCl_2$, (2) simultaneously with $CdCl_2$ and (3) 19 hours after administration of $CdCl_2$. The animals which received $Zn (CH_3CO_2)_2$ alone at this level (8 m. mol/kg.) the total amount was similarly

divided into 3 doses of 2·66 m. mol/kg. each. With the exception of this particular dose of Zn $(CH_3CO_2)_2$ (8 m. mol/kg.), the animals received $CdCl_2$ and Zn $(CH_3CO_2)_2$ (or SeO_2) simultaneously (Tables 1 and 2).

The males were sacrificed 168 hours and the females at two time intervals (48 and 168 hours) after injection of $CdCl_2$. The gonads and the accessory sexual organs were carefully dissected out and weighed to the nearest 0·1 mg. Pieces of tissues were fixed in Bouin's fluid and serial paraffin sections were stained with Ehrlich's hematoxylin followed by alcoholic eosin. The fructose concentration of the coagulating glands (CG) was estimated by the method given by Mann (1946).

RESULTS

I. *Effect of Zn $(CH_3CO_2)_2$ on $CdCl_2$ induced changes in the testis*

The testis weight was significantly reduced after administration of $CdCl_2$ (0·04 m. mol/kg.) (P < ·001). However, simultaneous administration of $CdCl_2$ and Zn $(CH_3CO_2)_2$ in equimolar dose caused a reduction of testis weight as compared to the normal controls (P < ·05). The loss of weight of the testis was also arrested when 200 times the previous dose of Zn $(CH_3CO_2)_2$ (8 m. mol/kg.) was given conjointly with $CdCl_2$ (0·04 m. mol/kg.). This high dose of Zn $(CH_3CO_2)_2$ alone did not evoke any statistically significant change in testis weight (Table 1).

The testis of animals injected with $CdCl_2$ (0·01–0·04 m. mol/kg.) presented hemorrhagic and atrophied condition. The seminiferous tubules were totally degenerated and the gametogenic elements appeared as an eosinophilic debris (Figs. 1 and 2). In most of the tubules the *tunica propria* was disintegrated. The remnants of dead spermatozoa were distinguishable in some tubules. The histological integrity of the interstitium was lost; and clumps of chromatin material extruded from the cytolysed cells were seen in this portion. The interstitial vessels were considerably engorged. The *tunica albuginea* was thickened.

Administration of $CdCl_2$ and Zn $(CH_3CO_2)_2$ in equimolar dose did not have any effect on histology of the testis; the picture was the same as in animals injected with $CdCl_2$ alone. When, however, the dose of Zn $(CH_3CO_2)_2$ was 200 times the dose of $CdCl_2$, the testis presented normal features (Fig. 3). Zinc acetate alone (0·04 and 8 m. mol/kg.) did not evoke any change in histology of the testis.

The seminal vesicles (SV) and the ventral prostate (VP) weights were significantly reduced after injection of $CdCl_2$ (0·01–0·04 m. mol/kg.) (P < ·001); the fructose concentration of CG either diminished to nothing (0·04 m. mol/kg.) or reduced significantly (0·01–0·02 m. mol/kg.) (P < ·001). The conjoint administration of $CdCl_2$ and Zn $(CH_3CO_2)_2$ in equimolar dose tended to stimulate the SV and VP weights (P < ·02–·01) and increase the fructose

TABLE 1

Antagonistic effect of zinc and selenium on cadmium with respect to male genital organs

Treatment	Testis weight (mg./100 gm. body weight) with S.E.	Seminal vesicle weight (mg./100 gm. body weight) with S.E.	Ventral prostate weight (mg./100 gm. body weight) with S.E.	Fructose concentration of the coagulating gland with S.E. (mg./gm.)
Normal controls*	736·43 ± 25·59 (6)*	68·82 ± 6·99 (6)	70·85 ± 6·54 (6)	0·215 ± 0·004 (6)
CdCl₂ (0·04 m. mol/kg.)	114·58 ± 7·73 (6)	7·00 ± 0·60 (6)	13·40 ± 0·87 (6)	0·000 ± 0·000 (6)
CdCl₂ (0·04 m. mol/kg.)+Zn (CH₃CO₂)₂ (0·04 m. mol/kg.)	719·93 ± 34·94 (6)	154·28 ± 3·72 (6)	89·52 ± 5·09 (6)	0·854 ± 0·078 (6)
Zn (CH₃CO₂)₂ (0·04 m. mol/kg.)+Zn (CH₃CO₂)₂ (8 m. mol/kg.)	628·20 ± 32·76 (6)	195·08 ± 14·56 (6)	109·45 ± 1·84 (6)	0·513 ± 0·063 (6)
CdCl₂ (0·04 m. mol/kg.)+Zn (CH₃CO₂)₂ (8 m. mol/kg.)	783·17 ± 46·60 (6)	100·45 ± 4·10 (6)	89·07 ± 3·45 (6)	0·345 ± 0·037 (6)
Zn (CH₃CO₂)₂ (8 m. mol/kg.)	667·87 ± 53·84 (6)	178·43 ± 6·29 (6)	96·63 ± 3·98 (6)	0·354 ± 0·040 (6)
CdCl₂ (0·04 m. mol/kg.)+SeO₂ (0·04 m. mol/kg.)	508·18 ± 46·64 (6)	140·57 ± 8·38 (6)	114·28 ± 5·72 (6)	0·581 ± 0·051 (6)
SeO₂ (0·04 m. mol/kg.)	815·88 ± 18·85 (6)	178·93 ± 9·10 (6)	100·75 ± 6·51 (6)	0·040 ± 0·002 (6)
CdCl₂ (0·02 m. mol/kg.)+SeO₂ (0·04 m. mol/kg.)	723·13 ± 41·69 (6)	45·67 ± 3·07 (6)	21·80 ± 1·31 (6)	0·431 ± 0·030 (6)
CdCl₂ (0·01 m. mol/kg.)+SeO₂ (0·02 m. mol/kg.)	615·02 ± 27·54 (6)	135·55 ± 3·81 (6)	23·97 ± 1·10 (6)	0·342 ± 0·017 (6)
SeO₂ (0·02 m. mol/kg.)	884·5 ± 20·10 (8)	124·00 ± 7·70 (8)	95·35 ± 6·10 (8)	0·271 ± 0·016 (8)
CdCl₂ (0·02 m. mol/kg.)	376·23 ± 29·01 (6)	16·63 ± 1·62 (6)	21·65 ± 0·94 (6)	0·070 ± 0·003 (6)
CdCl₂ (0·01 m. mol/kg.)	337·50 ± 10·22 (6)	33·68 ± 2·10 (6)	21·33 ± 1·52 (6)	0·130 ± 0·008 (6)

* Figure in parenthesis indicates the number of animals.

concentration of the CG (P<·001) (Table 1). Zinc acetate alone (0·04 m. mol/kg.) increased the SV and VP weights (P<·001); the CG fructose concentration too underwent a significant rise (P<·01) (Table 1). Such stimulation of SV and VP weights (P<·02-·01) and increase in fructose concentration of CG (P<·02) were noticed when Zn $(CH_3CO_2)_2$ was injected at a dose which was 200 times the dose of $CdCl_2$. Administration of this high dose of Zn (CH_3CO_2) alone caused a significant increase in weight of the accessory sexual organs (P<·02-·001) and concentration of fructose in the CG (P<·01).

II. *Effect of SeO_2 on $CdCl_2$ induced changes in the testis*

The weight of the testis of animals injected with $CdCl_2$ and SeO_2 conjointly (0·04 m. mol/kg.) was significantly greater than those treated with the former salt alone (P<·001). However, as compared to the normal animals, the testis weight of the combined group was significantly less (P<·01; Table 1). It was interesting that SeO_2 alone (0·02–0·04 m. mol/kg.) tended to increase the weight of the gland (P<·05-·02). When half the dose of $CdCl_2$ (0·02 m. mol/kg.) was administered along with SeO_2 (0·04 m. mol/kg.), the testis weight remained virtually unchanged. But a still further reduction in the level of $CdCl_2$ (0·01 m. mol/kg.) combined with half the dose of SeO_2 (0·02 m. mol/kg.) was not quite so effective because the weight of the testis continued to be low in comparison with that of the normal animals (P<·01; Table 1). The testis weight was reduced in animals injected with $CdCl_2$ alone in low doses (0·01–0·02 m. mol/kg.) (P<·001; Table 1).

Histologically, the testis of animals injected with $CdCl_2$ and SeO_2 in equimolar dose (0·04 m. mol/kg.) was like that of the group treated with the former alone (*see* Fig. 2). The macroscopic features too were reminiscent of typical $CdCl_2$ effects. In contrast, SeO_2 alone (0·02–0·04 m. mol/kg.) did not exert any harmful influence on the testis; the histological picture was essentially normal (*see* Fig. 1). When, however, the dose of $CdCl_2$ was halved (0·02 m. mol/kg.) but that of SeO_2 was kept as before (0·04 m. mol/kg.), the histological and macroscopic features of the testis were normal (Fig. 4). This was also the case when the dose of $CdCl_2$ was reduced to one quarter of the original dose (0·01 m. mol/kg.) and that of SeO_2 was halved (0·02 m. mol/kg.).

Administration of $CdCl_2$ and SeO_2 in equimolar dose (0·04 m. mol/kg.) caused a significant increase in SV and VP weights (P<·01-·001); the fructose concentration of the CG also underwent a rise (P<·001) (Table 1). The latter salt alone (0·04 m. mol/kg.) tended to stimulate the weight of these organs (P<·01-·001), but decrease the fructose concentration of the CG (P<·001; Table 1). Similar anomalous results were obtained when the dose of $CdCl_2$ was halved (0·02 m. mol/kg.) but that of SeO_2 was kept as before (0·04 m. mol/kg.). Thus, the SV weight was significantly reduced as compared with that of the normal control (P<·01), though it (SV weight) continued to

be higher than in $CdCl_2$ (alone) group (0·02 m. mol/kg.) (P<·001). In contrast, the VP weight and fructose concentration registered a significant rise (P<·001). It was interesting that administration of $CdCl_2$ (0·01 m. mol/kg.) and SeO_2 (0·02·m. mol/kg.) conjointly tended to stimulate the weight of these organs and increase the CG fructose (P<·05—·001). Selenium dioxide (0·02 m. mol/kg.) alone also caused an increase in weight of these accessories and concentration of fructose in the CG (P<·02—·001).

III.   *Effect of Zn $(CH_3CO_2)_2$ on $CdCl_2$ induced changes in the ovary*

Administration of $CdCl_2$ (0·04 m. mol/kg.—*48 hours*) caused a little, but significant increase in ovarian weight (*vs.* normal controls—*48 hours*, P<·001). In the case of animals injected with the same dose of the salt but sacrificed 168 hours later, the weight of the organ was significantly lower than that of the corresponding controls (*vs.* normal controls—*168 hours*, P<·01). Concurrent administration of $CdCl_2$ and Zn $(CH_3CO_2)_2$ in equimolar dose, the animals being killed *48 hours later*, did not cause any change in ovarian weight as compared with that of the animals injected with the former alone (*vs.* $CdCl_2$—0·04 m. mol/kg.—*48 hours*); though as compared with the weight of the corresponding controls that of the organ continued to be higher (*vs.* normal controls—*48 hours*, P<·001; Table 2). In contrast, the animals treated similarly with the two salts (0·04 m. mol/kg.), but killed *168 hours later*, had ovaries of almost similar weight as those of the $CdCl_2$ (0·04 m. mol/kg.—*168 hours*) group (Table 2). With zinc acetate alone (0·04 m. mol/kg.) in the case of animals killed *168 hours later* the ovaries were of almost similar weight to that of the $CdCl_2$ group (0·04 m. mol/kg.—*168 hours*; Table 2). Zinc acetate alone (viz. under the specific condition noted here 0·04 m. mol/kg.—*48 and 168 hours*) had little effect on ovarian weight. Even when the dose of Zn $(CH_3CO_2)_2$ was raised (8 m. mol/kg.) but that of $CdCl_2$ (0·04 m. mol/kg.) kept constant, the weight of the organ continued to remain unchanged as compared with that of the corresponding controls (*vs.* normal controls—*48 hours*; Table 2); but declined with respect to that of the $CdCl_2$ group (*48 hours*—P<·01). Similar assessment of the ovarian weight of animals injected with these salts ($CdCl_2$—0·04 m. mol/kg.+Zn $(CH_3CO_2)_2$—8 m. mol/kg.), and killed *168 hours later*, showed that it did not differ significantly either from that of the normal (*168 hours*) or the $CdCl_2$ controls (*168 hours*). Zinc acetate alone (8 m. mol/kg.) did not cause any significant change in the weight of the gland.

The histological examination of the ovary of control animals (*48 hours*) revealed immature or early puberal condition with primordial oocytes and young follicles in various stages of growth and also atresia (Fig. 5). The early stages of antrum formation was seen in some medium-sized follicles, but the occasional larger ones were anatomically more differentiated indicating
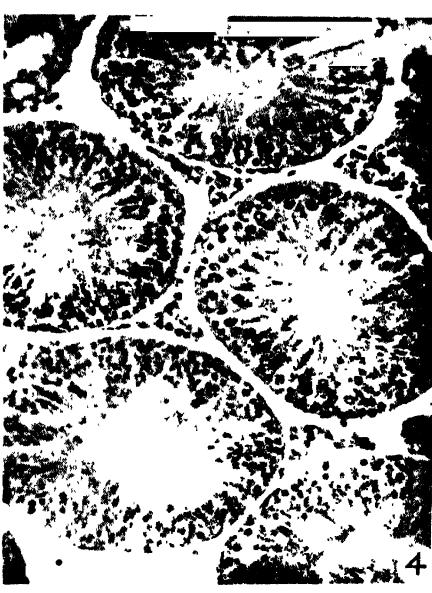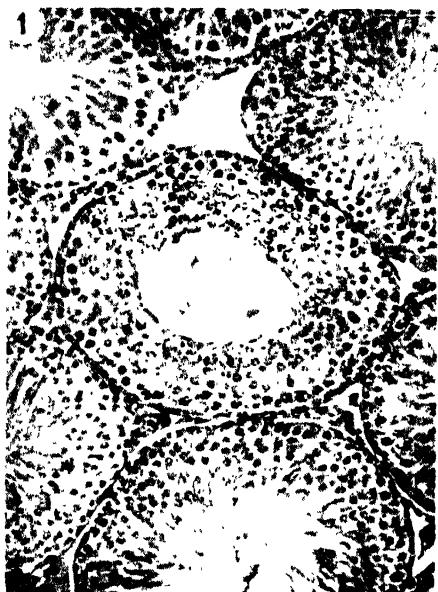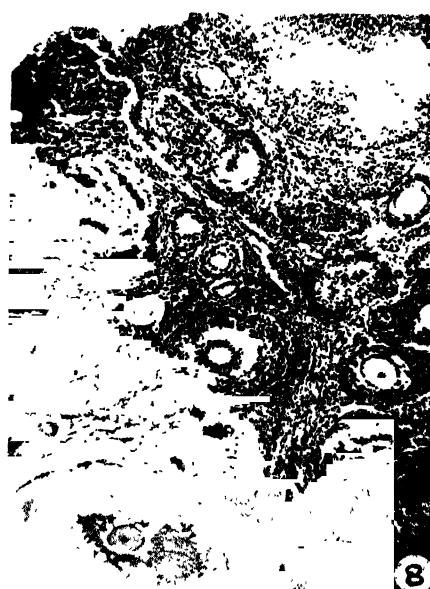
approaching maturity. The interstitium contained fibroblast-like and epithelioid elements with eosinophilic cytoplasm. The overall vascularity of the organ was poor, and there was no *corpus luteum* formation. In contrast, the ovary of the second group of control animals (*168 hours*) seemed to be more advanced as regards maturity. Thus, ripe follicles and spent ones in the process of *corpus luteum* formation were encountered in addition to oocytes and young follicles. *Corpora lutea* were present and, due to their bulk, the interstitium was considerably reduced (Fig. 6). The organ appeared to be more vascular also.

Macroscopically, the ovary of CdCl$_2$-treated animals (*48 hours*) was a hemorrhagic mass. The histological changes were similar to those described previously (Kar *et al.* 1959). These were essentially of a degenerative nature with mass atresia of the follicles, profuse hemorrhage, edema and widespread disorganization of the interstitium. The place occupied by the follicles appeared either as circular scar-like areas of yellow pigment with patches of an eosinophilic material, or as accumulations of degenerated granulosa cells (Fig. 7). In short, any semblance to a normal ovary was lost. However, the germinal epithelium and the contiguous oocytes appeared normal; even plaques of apparently healthy stromal tissue were encountered in the inner regions. There was extensive vascular engorgement particularly in the medullary portion. The histological features of the ovary of the other group of rats injected with CdCl$_2$ (*168 hours*) were similar to those of the corresponding normal controls (*168 hours, see* Fig. 6).

When CdCl$_2$ and Zn (CH$_3$CO$_2$)$_2$ were administered conjointly in equimolar dose (0·04 m. mol/kg.), and the ovaries examined histologically *48 hours later*, the features were like those of CdCl$_2$ (0·04 m. mol/kg.—*48 hours*) controls (*see* Fig. 7). Macroscopically too the ovary presented typical hemorrhagic appearance. But when the ovaries were studied *168 hours* after the administration of the two salts, essentially normal macroscopic and microscopic features were encountered (*see* Fig. 6). No degenerative changes were discernible in the ovary of animals injected with Zn (CH$_3$CO$_2$)$_2$ (0·04 m. mol/kg.) alone.

In contrast, the ovary of animals injected with a high dose of Zn (CH$_3$CO$_2$)$_2$ (8 m. mol/kg.) along with CdCl$_2$ (0·04 m. mol/kg.), presented typically normal features irrespective of the time interval (*48 hours* and *168 hours*) at which the animals were sacrificed. Such was also the case with the ovary of animals injected with the same high dose of the former salt alone.

The uterine weight of animals injected with CdCl$_2$ (0·04 m. mol/kg.), and sacrificed *48 hours later*, was significantly greater (P < ·001) than that of the corresponding normal controls (*48 hours*). But when a similar comparison was made between the other CdCl$_2$ group (*168 hours*) and the normal controls (*168 hours*), it was seen that the weight of the organ was significantly less in

the former group (P<·001; Table 2). The older controls (*168 hours*) had significantly heavier uterus than the other normal group (*vs.* control—*48 hours*;. P<·001). Zinc acetate (0·04 m. mol/kg.—*48 hours*) alone caused a significant increase in uterine weight as compared to *48 hours* controls (P<·001; Table 2); but animals sacrificed *168 hours* after the injection of the salt (0·04 m.·mol/kg.) did not differ significantly with their corresponding controls (*168 hours*) as regards the weight of the organ (Table 2). The high dose of Zn $(CH_3CO_2)_2$ (8 m. mol/kg.) tended to stimulate the uterine weight in the *48 hours* group (*vs.* normal controls—*48 hours*; P<·001), but reduce in the *168 hours* group (*vs.* normal controls—*168 hours*; P<·01). Combined administration of the two salts in equimolar dose (0·04 m. mol/kg.) increased the uterine weight over that of the $CdCl_2$ groups (P<·001) irrespective of the time when the animals were sacrificed (*48 or 168 hours*) (Table 2). Similar results were obtained when the comparison was made between the combined treatment groups (0·04 m. mol/kg.—*48 and 168 hours*) and the animals injected with Zn $(CH_3CO_2)_2$ (0·04 m. mol/kg.) alone (*48 and 168 hours*; P<·05–·001; Table 2). But the situation was different when similar assessment was made with the raised level of the latter salt (8 m. mol/kg.). Thus, animals injected with $CdCl_2$ (0·04 m. mol/kg.) and Zn $(CH_3CO_2)_2$ (8 m. mol/kg.) simultaneously, and sacrificed *48 hours later*, had significantly less uterine weight than did the corresponding $CdCl_2$ group (*48 hours*, P<·05); but the difference from the group treated with Zn $(CH_3CO_2)_2$ (8 m. mol/kg.) alone was negligible (Table 2). On the other hand, similar combined treatment with high dose of Zn $(CH_3CO_2)_2$ (8 m. mol/kg.), when the results were assessed *168 hours later*, showed an increase in uterine weight over that of the corresponding $CdCl_2$ (*168 hours*) or Zn $(CH_3CO_2)_2$ (alone) group (P<·01; Table 2).

IV. *Effect of SeO_2 on $CdCl_2$ induced changes in the ovary*

Injection of SeO_2 alone (*48 and 168 hours*) had no effect on ovarian weight of the treated animals when compared with that of the corresponding normal controls (*48 and 168 hours*). Combined administration of SeO_2 and $CdCl_2$ (*48 hours*) in equimolar dose (0·04 m. mol/kg.) tended to stimulate the weight of the organ over such weight in the case of groups treated with the two salts separately ($CdCl_2$—*48 hours*; SeO_2—*48 hours*) (P<·05–·02; Table 2). However, similar conjoint administration of the two salts and the assessment of ovarian weight after *168 hours* did not reveal any significant difference from such weight of the corresponding $CdCl_2$ (*168 hours*) or SeO_2 (*168 hours*) group (Table 2).

The histological features of the ovary did not change after administration of SeO_2 (*48 or 168 hours*). Similarly, the combined injection of SeO_2 and $CdCl_2$ in equimolar dose (0·04 m. mol/kg., *48 and 168 hours*) was without

TABLE 2

Antagonistic effect of zinc and selenium on cadmium with respect to female genital organs

Treatment	Ovarian weight (mg./100 gm. body weight) with S.E.	Uterine weight (mg./100 gm. body weight) with S.E.
Normal controls—*48 hours*	10·48 ± 0·40 (6)*	21·20 ± 1·01 (6)
CdCl₂ (0·04 m. mol/kg.)—*48 hours* ..	14·15 ± 0·59 (6)	40·17 ± 2·07 (6)
CdCl₂ (0·04 m. mol/kg.) + Zn (CH₃CO₂)₂ (0·04 m. mol/kg.)—*48 hours* ..	14·63 ± 1·31 (6)	64·38 ± 3·23 (6)
Zn (CH₃CO₂)₂ (0·04 m. mol/kg.)—*48 hours* ..	10·67 ± 0·80 (6)	33·25 ± 1·08 (6)
Normal controls—*168 hours*	18·33 ± 0·96 (6)	79·78 ± 1·54 (6)
CdCl₂ (0·04 m. mol/kg.)—*168 hours* ..	11·37 ± 0·42 (6)	46·02 ± 1·54 (6)
CdCl₂ (0·04 m. mol/kg.) + Zn (CH₃CO₂)₂ (0·04 m. mol/kg.)—*168 hours*	15·40 ± 1·40 (6)	91·92 ± 3·02 (6)
Zn (CH₃CO₂)₂ (0·04 m. mol/kg.)—*168 hours*	16·22 ± 0·26 (6)	70·65 ± 6·55 (6)
CdCl₂ (0·04 m. mol/kg.) + Zn (CH₃CO₂)₂ (8 m. mol/kg.)—*48 hours* ..	10·70 ± 0·54 (6)	31·87 ± 0·91 (6)
Zn (CH₃CO₂)₂ (8 m. mol/kg.)—*48 hours* ..	13·35 ± 0·97 (6)	36·83 ± 3·64 (6)
CdCl₂ (0·04 m. mol/kg.) + Zn (CH₃CO₂)₂ (8 m. mol/kg.)—*168 hours* ..	14·83 ± 1·21 (6)	63·87 ± 1·74 (6)
Zn (CH₃CO₂)₂ (8 m. mol/kg.)—*168 hours* ..	14·53 ± 0·72 (6)	38·95 ± 1·15 (6)
CdCl₂ (0·04 m. mol/kg.) + SeO₂ (0·04 m. mol/kg.)—*48 hours* ..	17·03 ± 1·15 (6)	75·05 ± 1·82 (6)
SeO₂ (0·04 m. mol/kg.)—*48 hours* ..	11·00 ± 0·67 (6)	32·37 ± 0·91 (6)
CdCl₂ (0·04 m. mol/kg.) + SeO₂ (0·04 m. mol/kg.)—*168 hours* ..	12·88 ± 1·54 (6)	38·75 ± 1·33 (6)
SeO₂ (0·04 m. mol/kg.)—*168 hours* ..	10·50 ± 0·35 (6)	26·65 ± 1·04 (6)

* Figure in parenthesis indicates the number of animals.

any effect on the histology of the organ; typically normal features were consistently encountered (Fig. 8).

The uterine weight did not change significantly after injection of SeO_2 (*48 hours*) (*vs.* normal controls—*48 hours*). But animals treated with the salt, and sacrificed *168 hours* after, had significantly smaller uteri than did the corresponding normal controls (*168 hours*; P<·001). Combined administration of SeO_2 and $CdCl_2$ (*48 hours*) was associated with a significant increase in weight of the organ over that of the groups treated with the two salts separately (SeO_2—*48 hours* and $CdCl_2$—*48 hours*) (P<·001; Table 2). However, the animals treated with the two salts together, and sacrificed *168 hours* afterwards, had significantly lower uterine weight than did the corresponding $CdCl_2$ (*168 hours*) group (P<·01); but the weight was higher than what the SeO_2 (*168 hours*) group (P<·01; Table 2) showed.

DISCUSSION

The results obtained in the present study bear out the findings of Parizek (1957) regarding prevention by Zn of degenerative changes in the rat testis induced by $CdCl_2$. In addition, it has been possible to demonstrate a similar antagonistic effect of Zn on Cd with respect to ovary. An interesting point of similarity between the sexes is that a relatively high dose of Zn $(CH_3CO_2)_2$ (200 times the equimolar dose of $CdCl_2$) is necessary to prevent the gonadal changes caused by $CdCl_2$; administration of the two salts in equimolar dose (0·04 m. mol/kg.) is ineffective as the gonads are promptly atrophied in response to $CdCl_2$. The dosage factor is important because it may have a bearing on the mechanism by which the gonadal degeneration is prevented by Zn.

It is difficult to correlate the changes in weight with the histological picture of the testis. Thus, the loss of weight of this organ caused by $CdCl_2$ is prevented by Zn $(CH_3CO_2)_2$ irrespective of the dosage in which it is injected. As mentioned above, this is not the case with the microscopical features which are preserved like normals only, when the dose of Zn is considerably higher than that of Cd. Similarly, Zn alone tends to reduce the weight without exerting any untoward effects on histology of the testis. Curiously, the weight of the accessory sexual organs is significantly increased by Zn $(CH_3CO_2)_2$ whether given alone or in combination with $CdCl_2$. Such a consistent stimulatory effect is also exerted on fructose concentration of the CG. These effects of Zn $(CH_3CO_2)_2$ are difficult to interpret because, even in animals injected with this salt alone, there is no indication whatsoever of any morphological change in the Leydig cells. Therefore, it can only be conjectured that Zn either increases the sensitivity of the accessory sexual organs to androgen, or reduces its (androgen) rate of metabolic degradation. In fact, Zn has been reported to augment the action of testosterone on the accessory genital organs (Urbain *et al.* 1938). Nevertheless, these arguments do not

satisfactorily explain this anomalous effect of Zn $(CH_3CO_2)_2$ particularly when infused with $CdCl_2$ (in equimolar dose), because animals so treated have no functional Leydig cells in their defunct testis. Whether in these animals the output of adrenocortical androgens is increased by Zn $(CH_3CO_2)_2$ cannot be hazarded from the present meagre data.

In case of SeO_2 also the dosage has to be higher than $CdCl_2$ in order to prevent the characteristic degenerative changes in the testis. Equimolar dose of SeO_2 (0·04 m. mol/kg.) is ineffective in this respect in spite of the fact that the loss of testis weight, seen in $CdCl_2$ groups, is prevented to a large extent. It is interesting that SeO_2 alone tends to increase the testis weight without commensurately influencing the histology of the organ. The effect of this salt (either alone, or in combination with $CdCl_2$) on the accessory sexual organs is anomalous and of doubtful significance in relation to its salutary effect on the testis.

In contrast, an equimolar dose of SeO_2 (0·04 m. mol/kg.) is sufficient to prevent the ovarian changes caused by $CdCl_2$; although like males, a considerably high dose of Zn $(CH_3CO_2)_2$ is needed to oppose the effect of $CdCl_2$. The two salts themselves (SeO_2 and Zn $(CH_3CO_2)_2$) do not, however, cause any change in the histology of the ovary. Ponderal changes of one type or the other are, of course, seen in the organ but these do not seem to bear any relationship to the histological changes. Similarly, in the present context, little importance can be attached to the anomalous fluctuations in uterine weight.

The mechanism responsible for the effect of Cd on gonads is mostly unknown. However, Kar et al. (1959; Kar and Das 1960; Kar et al. 1960) have shown that the gonadotrophic hormonal activity is interrupted both at the level of the pituitary and the gonads. There is also a drastic inhibition of succinoxidase activity in the gonads (Kar, unpublished) but the store of vitamin E (Kar, unpublished) and sulphydril groups (Parizek 1957) remains unaffected. These findings may be pertinent, but not adequate, to explain such prompt and total degeneration of gonads by Cd; consequently, the mechanism responsible for the antagonistic effect of Zn and Se has to be considered against a background which is not quite clear.

Parizek (1957) believes that Cd acts by a displacement of Zn from spermatogenic epithelium which is particularly rich in this metal (Zn) (Mawson et al., 1955). In point of fact, it has been demonstrated that inadequate nutritional uptake of Zn leads to degeneration of the spermatogenic tubules, indicating the important role of this metal in spermatogenesis (Elcoate et al. 1955). Studies on yeast too have shown that the inhibitory effect of Cd on the growth of these micro-organisms can be antagonized by Zn (White and Munus 1951). Further, the close physicochemical similarities between Zn and Cd make such a competitive antagonism between them seem likely.

4

Nevertheless, this explanation does not rest on very firm grounds because, contrary to expectations, the effect of Zn appears to be nonspecific; Se also antagonizes the action of Cd on the gonads. Besides, there are no data to indicate that Se like Zn is a normal constituent of the spermatogenic epithelium, and as such it is difficult to conceive that a Se-deficiency state is produced in the testis by a displacement of this metal (Se) by Cd. But in case this is so, it would mean (by analogy with Zn) that simultaneous administration of Se simply overcomes this deficiency resulting in continuation of normal spermatogenesis. It is, however, possible that Zn and Se antagonize Cd *at two different sites*; Zn at the level of testis but Se at a site *away from this organ* (circulation ?). Whatever may be the case, the possibility remains that Cd is somehow removed by Zn and Se from these sites; Se seems to be more effective because it can act at the equimolar dose (at least in females), whereas a considerably higher dose of Zn is needed for antagonizing Cd effects.

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EXPLANATION OF FIGURES (PLATES VI AND VII)

(All figures are photomicrographs and are magnified × 170)

1. Testis of a normal rat. Note full spermatogenesis and active Leydig cells in the interstitium.
2. Testis of a $CdCl_2$ (0·04 m. mol/kg.) treated rat. Note extensive degeneration of the seminiferous epithelium and the interstitial elements.
3. Testis of a rat injected with $CdCl_2$ (0·04 m. mol/kg.) and Zn $(CH_3CO_2)_2$ (8 m. mol/kg.) simultaneously. Note normal condition of the testis.
4. Testis of rat treated with $CdCl_2$ (0·02 m. mol/kg.) and SeO_2 (0·04 m. mol/kg.) simultaneously. Note normal features of the testis.
5. Ovary of a normal rat (*48 hours*). Note prepuberal condition of the ovary.
6. Ovary of a normal rat (*168 hours*). Note large follicle and *corpus luteum* (lower bottom of the figure).
7. Ovary of a $CdCl_2$ treated rat (*48 hours*). Note destruction of the follicles and the interstitium.
8. Ovary of $CdCl_2$ plus SeO_2 (0·04 m. mol/kg., *48 hours*) treated rat. Note normal features.

4B

ECOLOGICAL DISTRIBUTION OF *PERISTROPHE BICALYCULATA* NEES.

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ABSTRACT

Peristrophe bicalyculata Nees., an annual tall herb, grows on nitrogen rich soil in shade and on nitrogen poor soil in open. Field studies supported by culture experiments show that germination of seeds and growth respond effectively to such combination of the soil and light factors. Chemical analysis of soils and plants indicates higher uptake of nitrogen in shade; but deep shade proves to be detrimental to growth in as much as carbon assimilation suffers and nitrogen accumulates in the tissues. The significance of these investigations on the ecology of the plant is discussed.

Peristrophe bicalyculata Nees. (Synonym: *Justicia bicalyculata* Vahl.) is an erect spreading annual herb which grows 80–125 cm. tall as a weed in waste places. It is confined to the warmer parts of India with extensions in tropical Africa and Afghanistan. Preliminary observations reported elsewhere (Misra and Ramakrishnan 1959) showed that the species is found in small colonies either on organic soil under the shade of trees or on nitrogen poor sandy soil in the open. This ecological behaviour of the plant prompted probing into its biological equipment for compensatory actions of light intensity and soil nitrogen.

Characters of the soil for the incidence of the species are set in Table I. The colonies thrive on drier soils and are not found in moist or waterlogged habitat. The moisture range found is 3·3 per cent to 9·3 per cent of the soil dry-weight. The reaction of the soil is either alkaline or neutral with 0·05 to 8·56 per cent of carbonates and 9·2 to 42·0 m.e. per cent of exchangeable calcium. Thus the plant grows both on calcareous and non-calcareous soils. Nitrate nitrogen estimates give a range of 2·10 to 2·80 mg./100 g. of soil in the open and 3·00 to 6·13 mg./100 g. of soil in partial shade. Organic content varies from 2·4 to 11·0 per cent.

An examination of the plant characters in relation to the habitat revealed the following facts:—

Seed output: The average seed output per plant from exposed and shaded colonies is given in Table II from which it will be seen that exposed plants give a higher yield. The seeds are flat and orbicular with a notch on one side. They are 1·775 to 2·359 mm. long and 1·506 to 1·955 mm. broad giving a shape index (length/breadth) of 0·9 to 1·3, and weigh about 1·51 mg. each, irrespective of exposure and edaphic factors.

TABLE I

Soil analysis data for P. bicalyculata Nees.

Locality	Moisture content[1] (% of dry wt.)		pH[2]		Carbonate content[3] (%)		Exchangeable Calcium[4] (m.e. %)		Nitrate Nitrogen[5] (mg./100 g. of soil)		Organic matter[6] (%)	
	Open	Partial shade	Open	Partial shade	Open	Partial shade	Open	Partial shade	Open	Partial shade	Open	Partial shade
Latifshaw	4·1	8·4	8·2	8·0	8·56	2·86	42·0	35·0	2·60	3·40	2·8	4·6
Rajghat	3·9	5·4	8·5	8·5	1·55	2·26	29·2	30·9	2·20	3·00	2·4	6·8
Sarnath	5·7	9·3	6·9	7·4	0·17	0·33	19·4	18·7	2·80	3·80	4·2	5·3
University area	3·3	5·7	7·5	7·9	1·99	0·08	39·2	9·2	2·48	3·75	2·7	2·9
Ramnagar	3·8	9·8	7·5	7·5	5·12	3·28	21·1	41·2	2·75	6·13	3·1	11·0
Akbari	4·2	6·9	7·4	7·5	2·82	0·05	28·0	10·1	2·10	4·10	2·6	4·0

[1] Determined by oven drying at 105° C.   [2] Electrometrically with a photovolt pH meter.   [3] Hutchinson and McLenan's method as outlined by Piper (1944).   [4] Leaching with N/2 acetic acid, Wright (1939).   [5] Snell and Snell (1949)—colorimetrically using Klett Summerson photoelectric colorimeter.   [6] Robinson's method—*see* Wright (1939).

TABLE II

Seed output of P. bicalyculata *Nees.*

Locality	Average seed output per plant	
	Open	Partial shade
Latifshaw ..	2,319	1,082
Rajghat ..	8,497	457
Sarnath ..	1,677	1,663
University area ..	1,688	1,182
Ramnagar ..	1,288	920
Akhari ..	2,489	777
Average ..	2,993	1,014

Dispersal of seeds: The valves of the capsule suddenly separate and the retinaculum springs up throwing the seed with jerk at some distance. The effectiveness of the explosive mechanism has been studied by putting mature capsules in the centre of concentric circles drawn on paper and then noting the frequency of seeds reaching different distances after 7 hours. The results are plotted in Text-fig. 1 from which it will be seen that majority of the seeds are thrown to a distance of 10-15 cm. which, of course, will be longer from the height of the plant. Further carriage of seeds by wind is highly probable in view of their lightness.

Germination: Seeds collected on January 26, 1958, from a uniform population were used in experiments. Fresh seeds gave a percentage germination of 6 which increased to 28 after 35 days and to 32 after seven months' dry storage. Intensity and duration of light also affect percentage germination as can be seen from the data set in Table III. Light as such promotes germination and in continuous light the maximum percentage of germination is obtained. However, germination in relation to nitrate and light is still more interesting. In these experiments germination was tried in soil with additions of varying doses of ammonium nitrate in separate pots which were placed in three sets in the open, partial shade and deep shade respectively. The results are given in Table IV. From the data it is obvious that ammonium nitrate in the soil is in some way compensatory to light intensity in the germination of seeds of *Peristrophe bicalyculata* Nees. The nitrate inhibits germination in strong light and promotes it in weak light.

Reproductive and aggressive capacities: Salisbury (1942) defines reproductive capacity as the product of the seed output and the fraction representing the percentage germination. It gives the pressure of plant regeneration in a habitat. When the figure for reproductive capacity is multiplied with the

NO. OF SEEDS DISPERSED
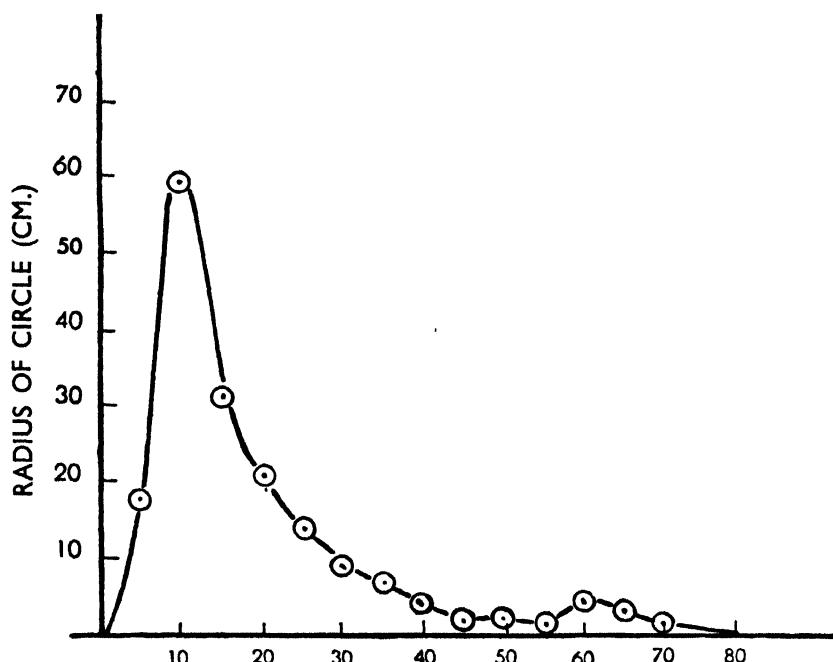
TEXT-FIG. 1. Graph showing dispersal of seeds of *Peristrophe bicalyculata* Nees. at various distances.

TABLE III

Germination of seeds of P. bicalyculata *Nees. under different light conditions*

Date	Diffused daylight from window	Continuous electric light	Continuous darkness
21–7–58 ..	0	4	0
22–7–58 ..	2	2	1
23–7–58 ..	4	7	1
24–7–58 ..	2	4	0
25–7–58 ..	1	6	2
26–7–58 ..	1	2	1
27–7–58 ..	0	2	1
29–7–58 ..	0	2	0
30–7–58 ..	6	0	0
Percentage germination ..	32	58	12

TABLE IV

Germination of seeds of Peristrophe bicalyculata *Nees. in soil with additions of ammonium nitrate under three exposures of light condition*

Amount in g. of ammonium nitrate added to the soil	Percentage germination in		
	Open	Partial shade	Deep shade
0·0	16	0	0
0·2	16	16	16
0·4	0	12	20
0·6	0	8	20
0·8	0	4	40

percentage survival of seedlings, the product becomes the aggressive capacity of the species in the habitat. These figures are valuable indices in deciding the dominance of a species within the plant community. The data for *Peristrophe bicalyculata* Nees. are given in Table V from which it will be seen that the lower seed output is compensated by the higher percentage survival in partial shade so that the aggressive capacity of the plant remains the same under the two light conditions. The actual figures for percentage survival of seedlings are recorded in Table VI.

TABLE V

Reproductive capacity and aggressive capacity of P. bicalyculata

Exposure	Seed output	Percentage germination	Reproductive capacity	Percentage survival	Aggressive capacity
Open	2,993	32	957	9	86
Partial shade	1,013	32	324	27	87

Seedling morphology: Germination is epigeal. The radicle comes out from just below the notch of the seed. The hypocotyl elongates as a hook taking up the cotyledons together with the testa. The first pair of leaves differs from the leaves of the adult plant in size and shape. The different stages are drawn in Text-fig. 2.

Epidermal structure and stomatal frequency of leaves: The leaves are hairy above and densely so on the veins beneath. Stomata are present on both the surfaces. The epidermal cells are more wavy in surface view and the number of stomata per unit area is higher on the lower side. Further, stomatal density is higher in the open. The measurements are given in Table VII.

TABLE VI

Percentage survival of P. bicalyculata *Nees. in relation to exposure*

Date	Number of seedlings in the quadrat				
	Open		Partial shade		Deep shade
	Rajghat I	Univ. area II	Rajghat I	Univ. area II	Rajghat I
15–7–58 ..	62	98	122	107	87
15–8–58 ..	20	29	63	51	12
15–9–58 ..	6	8	29	32	0
Percentage survival ..	9·7	8·2	23·8	29·9	nil

TEXT-FIG. 2.   Stages in the germination of seeds of *Peristrophe bicalyculata* Nees.

TABLE VII

Stomatal frequency and stomatal index in 'sun' and 'shade' leaves of P. bicalyculata Nees.

Sl. No.	Upper epidermis		Lower epidermis		Stomatal index	
	Stomata per sq. mm.	Epidermal cells per sq. mm.	Stomata per sq. mm.	Epidermal cells per sq. mm.	Upper surface	Lower surface
Sun 1	147	441	225	549	25	29
2	147	471	275	657	24	30
3	147	422	255	637	26	26
Shade 1	49	284	118	441	15	21
2	39	235	127	461	14	22
3	39	245	127	441	14	22

Osmotic pressure of the plant sap: Cryoscopic determinations of the osmotic pressure are given in Table VIII in relation to soil moisture. The figures and range of variations are those of a typical mesophyte.

TABLE VIII

Osmotic pressure of plant sap of P. bicalyculata Nees.

Locality	Moisture content in the soil (%)	Osmotic pressure of the plant sap (atmos.)
I	2·3	20·129
II	4·9	18·839
III	13·1	13·118

A correlation could be established between moisture content in the soil and the osmotic pressure of the plant sap. The osmotic pressure values range well within that of mesophytes.

Phenology: Seedlings appear in nature by the middle of July and can be collected up to the end of August. Flowering starts by the end of September and continues with fruit development up to March when the plants dry up.

Biotic factors: The associates of *Peristrophe bicalyculata* Nees. are given in Table IX. The plants are grazed by cattle and the following fungal parasites on this species are reported in India according to the information received from the Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi:—

1. *Cercospora peristrophes* on leaves.
2. *Cercosporella peristrophes* on leaves.

3. *Exotrichum leucomelas* on leaves.
4. *Plasmopara wildermaniana* on leaves.
5. *Ramularia* sp. on leaves.
6. *Synchytrium rytzii* on leaves, stems and petioles.

Nitrogen content of plants: Soil and plants collected from exposed and partially shaded situations were analysed for nitrate and total nitrogen contents respectively. The data are set in Table X. There is clear indication of high nitrogen requirement and utilization by the plant in shade.

TABLE IX

Associates of P. bicalyculata *Nees. in open* (O.) *and partial shade* (P.S.)

Species	1 O.	1 P.S.	2 O.	2 P.S.	3 O.	3 P.S.	4 O.	4 P.S.	5 O.	5 P.S.	6 O.	6 P.S.
Peristrophe bicalyculata	a	f	v.f	v.f	a	f	o.f	f	o.f	f	o.f	a
Achyranthes aspera	o.f	o.f	f	f	f	a	f	- -	o.f	a	r	f
Aerua caudata	—	—	—	r	—	—	—	—	—	—	—	—
Amarantus viridis	—	—	—	—	—	—	—	- -	- -	r	—	—
Argemone mexicana	o.f	—	—	—	—	—	- -	—	—	—	f	—
Azadirachta indica (seedlings)	—	—	f	—	—	—	- -	—	—	—	—	—
Blepharis sp.	—	—	o.f	—	—	—	—	—	—	—	—	—
Bothriochloa pertusa	—	—	—	- -	—	- -	—	o.f	—	—	- -	—
Calotropis procera	o.f	- -	- -	- -	—	-	—	—	—	—	—	—
Cassia occidentalis	—	—	r	o.f	—	—	—	—	—	—	—	—
Corchorus acutangularis	- -	—	r	- -	—	—	—	- -	—	—	—	—
Cynodon dactylon	—	—	—	—	—	—	—	- -	a	o.f	- -	—
Datura sp.	- -	—	r	—	—	—	—	—	—	—	—	—
Desmodium gangeticum	—	o.f	- -	r	—	r	—	—	- -	—	—	—
Dichanthium annulatum	a	—	—	—	o.f	o.f	—	f	—	- -	f	o.f
Euphorbia hirta	f	r	—	—	r	—	o.f	—	—	- -	o.f	f
Evolvulus alsinoides	—	—	—	—	—	—	r	- -	—	—	—	- -
Justicia indica	—	—	—	r	—	—	- -	—	—	—	—	- -
J. quinqueangularis	—	- -	—	- -	—	- -	—	r	—	- -	—	- -
Launea asplenifolia	r	- -	- -	—	—	—	o.f	- -	- -	—	—	- -
Linaria ramosissima	—	- -	- -	v.f	- -	—	—	—	—	- -	- -	- -
Nepeta ruderalis	—	—	—	o.f	—	f	—	o.f	—	—	- -	—
Ocimum canum	f	- -	—	—	—	—	—	r	—	—	—	—
Rhynchosia minima	—	- -	—	—	—	- -	o.f	r	—	o.f	- -	—
Rungia parviflora	—	- -	—	—	—	—	—	—	- -	r	—	f
Scoparia dulcis	—	—	—	—	—	—	—	r	—	—	—	—
Sida veronaecifolia	—	—	o.f	—	—	—	—	—	—	—	—	—
Solanum xanthocarpum	f	- -	—	—	—	—	—	—	—	—	—	—
Themeda caudata	—	—	—	f	—	—	—	—	—	—	—	—
Tridax procumbens	f	o.f	—	—	—	—	—	—	—	—	—	o.f
Triumfetta neglecta	—	—	—	—	—	—	—	r	—	—	—	—
Vernonia cinerea	—	—	—	—	—	—	—	f	—	—	—	—

* Localities:—1, Latifshaw; 2, Rajghat; 3, Sarnath; 4, University area; 5, Ramnagar; 6, Akhari.
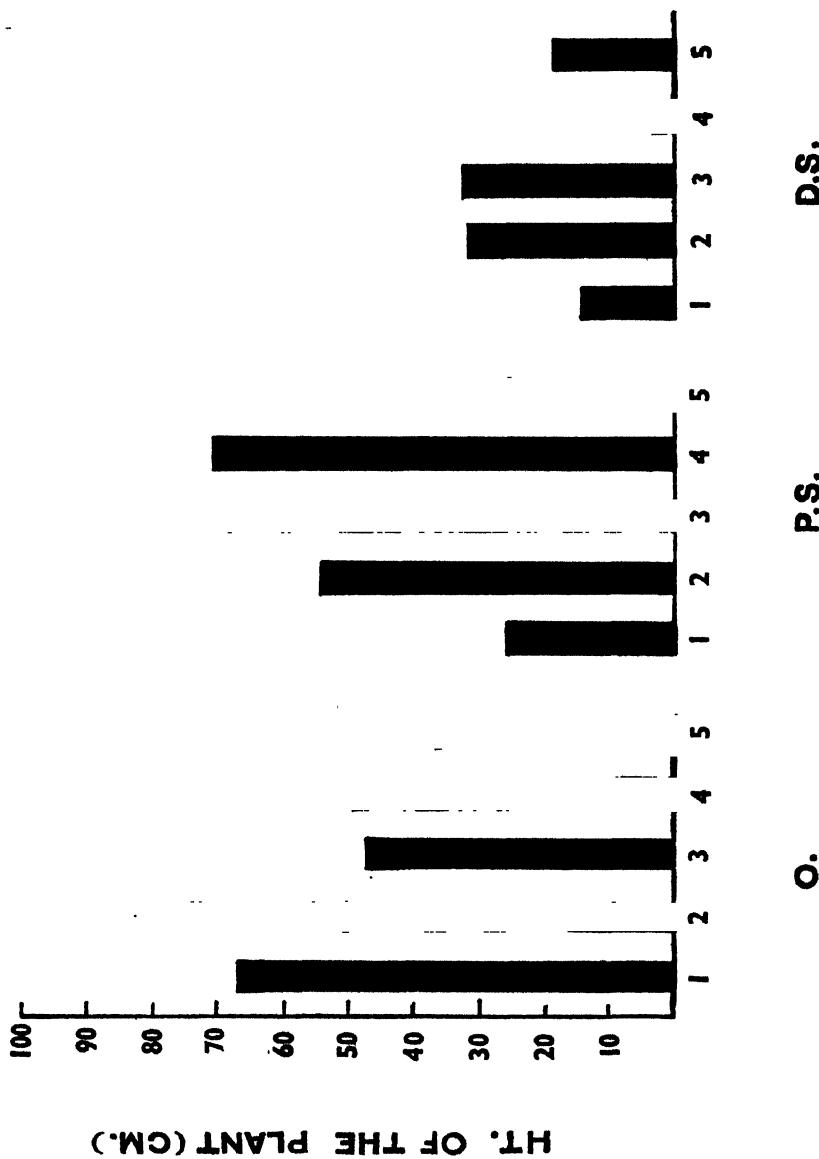
TEXT-FIG. 3. Height of plants of *Peristrophe bicalyculata* Nees. grown in soils treated with different monthly doses of ammonium nitrate in open (O.), partial shade (P.S.) and deep shade (D.S.). 1, control; 2 to 5 represent monthly treatments of 0·2, 0·4, 0·6 and 0·8 g. respectively of ammonium nitrate.
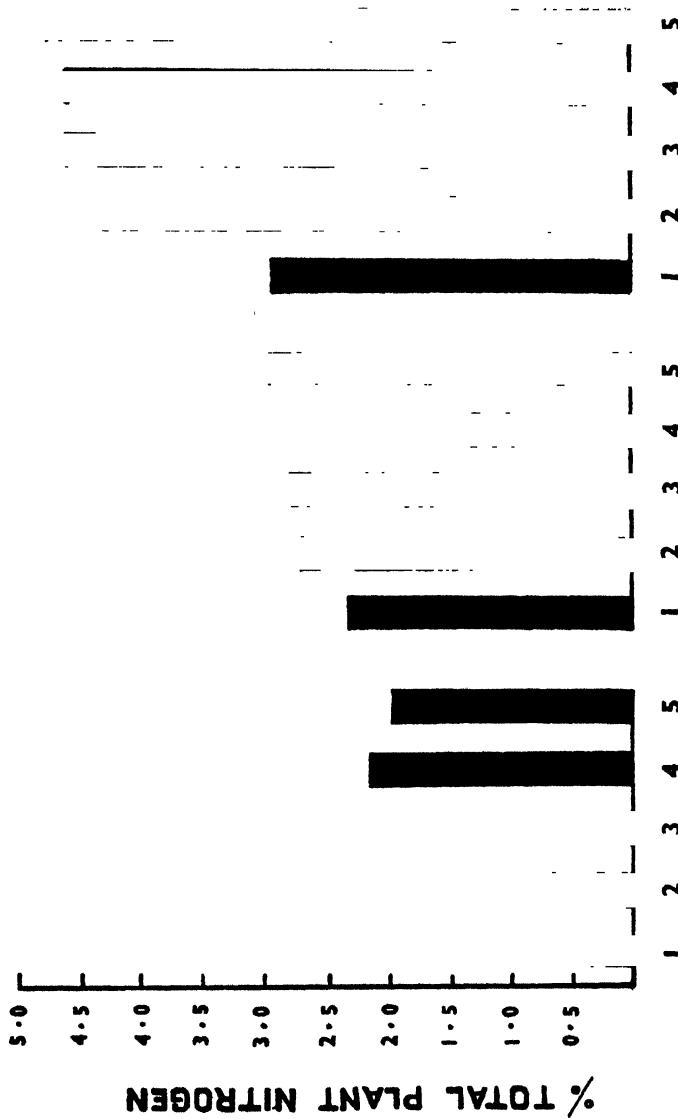
TEXT-FIG. 4. *Peristrophe bicalyculata* Nees. relationship between total plant nitrogen and soil nitrate in open (O.), partial shade (P.S.) and deep shade (D.S.). 1, control; 2 to 5 represent monthly treatments of 0·2, 0·4, 0·6 and 0·8 g. respectively of ammonium nitrate.

TABLE X

Total nitrogen content of P. bicalyculata *and nitrate nitrogen content of the soil*

Locality		Nitrate nitrogen in soil (mg./100 g. of soil)		Total nitrogen in plant (%)	
		Open	Partial shade	Open	Partial shade
Latifshaw ..	..	2·60	3·40	2·80	3·24
Rajghat ..	..	2·20	3·00	2·60	3·08
Sarnath ..	..	2·80	3·80	2·94	4·83
University area	..	2·48	3·75	3·00	3·71
Ramnagar	..	2·75	6·13	3·01	4·91
Akhari ..	..	2·10	4·10	2·48	3·87

Culture experiments: Three sets of five pots each with monthly additions of different doses of ammonium nitrate were prepared with garden soil. Set A pots were placed in the open, set B in partial shade and set C in deep shade. Two thrifty and identical seedlings were planted in each pot and the growth was harvested separately after three months for analysis of plant nitrogen. The growth performance of the plants with varying doses of soil nitrate under three light exposures is shown in Pl. VIII, Figs. 1–3 and Text-fig. 3. The nitrogen content of the corresponding harvests is shown in Text-fig. 4. From these it is evident that the best performance is found under partial shade with higher doses of nitrate in the soil. In deep shade growth suffers considerably in spite of enhanced nitrogen absorption.

DISCUSSION

Comparatively very little work has been done in the study of the uptake of nutrients and interaction of light. Pagnoul (1879, 1881) and Thatcher (1909) found that shading of the plants resulted in an increase of the ash content of the plants. According to Kraybill (1923) shading with a layer of cotton cloth increased the percentage nitrogen content of shoots of both apples and peaches. Mitchell (1934) found that shading of *Pinus sylvestris* did not suppress the uptake of nitrogen except when the level of nitrogen in the soil is high. Gast (1937) linked the effect of lower light intensity in depressing the absorption of nitrogen in *Pinus sylvestris* with the availability of nitrogen in the medium, since the greater the nitrogen availability in pot culture medium, the greater the depression of nitrogen uptake. On the other hand, the percentage nitrogen content of the plants rose as the light intensity fell. Blackman and Rutter (1946, 1947, 1948, 1949) have also investigated this aspect, in detail, for *Scilla non-scripta*.

From these investigations, it is seen that light influences the uptake of nutrients. The present experiments also confirm such an interaction between light intensity and uptake of nitrogen in *Peristrophe bicalyculata* Nees.

The natural distribution of *P. bicalyculata* Nees. in nitrogen poor soil in the open and nitrogen rich soil in shade is explained primarily on account of most favourable germination of seeds and subsequent normal growth in partial shade. A comparative study of soil nitrate and total plant nitrogen gives higher values for the latter in shade. With an increase in nitrate content of the soils, values for plant nitrogen decrease in the open while they increase in partial shade. In deep shade, total plant nitrogen values are enormously high which result in deranged nitrogen metabolism leading to poor growth.

Blackman and Rutter (1949) find that lowering of the light intensity tends to an increase in the total amount of nitrogen in the shoots though they find that the overall absorption is reduced under this condition. Such accumulation implies that the nutrients are in excess and cannot be utilized for growth due to limited carbohydrate supply. This does not indicate, however, that there is an internal surplus in shaded plants. Nevertheless, there is evidence to show that unelaborated nitrogen accumulates in the leaves of shaded grasses (Blackman and Templeman, 1940).

In *P. bicalyculata* Nees. the problem of interaction of light and nitrogen appears to be somewhat peculiar. Blackman and Rutter (1946, 1947, 1948, 1949) worked with a perennial bulbous monocotyledon (*Scilla non-scripta*) where there is a possibility of storage of nitrogen in the bulb. Therefore, they show that in low light intensities, the increase in nitrogen content of the shoot is small compared to the loss of this element in the bulb. Thus they concluded that nitrogen absorption is depressed by shading. In the present instance, there is not much possibility of an accumulation of nutrients in the roots as it is not a storage organ. Moreover, the decrease in total nitrogen content with increase in the level of soil nitrogen in the open, and the reverse in partial shade and deep shade do not yield to any simple explanation.

Blackman and Rutter (1947) have observed that lowering of light intensity also tended to depress seed production. In *P. bicalyculata* Nees., such a phenomenon appears to be partly due to low light intensity and also due to increase in soil nitrogen. To conclude with, light intensity along with nitrogen nutrition appears to play an important rôle in the distribution of the plant.

Hanson (1958) points out that every species has a certain capacity or efficiency in utilizing the available resources of the habitat in which it occurs. Whitehead (1954, 1956) recognizes that this capacity or efficiency is the result of the genetical potential and effectiveness of physiological processes. Thus, *P. bicalyculata* Nees., as shown in the present study, is able to utilize the available nitrogen from a nitrogen poor soil in the open and nitrogen rich soil in partial shade. In this case, the effectiveness of the physiological processes
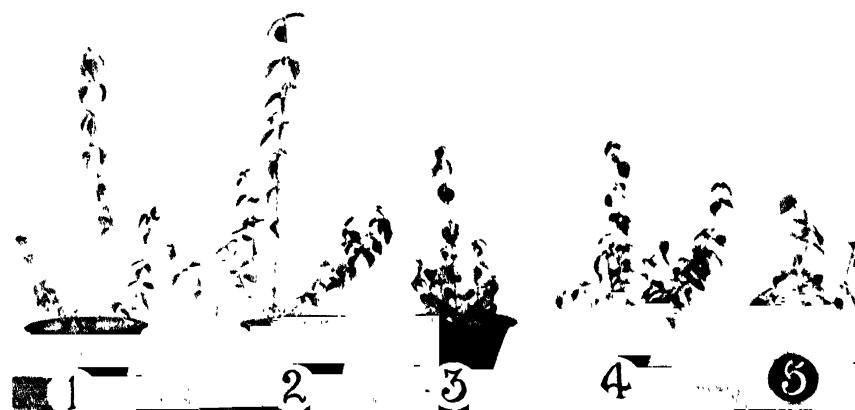
Explanation of Plate Figures

FIG. 1   *Perestropha bicalyculata* Nees plants growing in open in soils treated with different doses of ammonium nitrate.
    FIG. 1.1 —control; FIGS. 1.2 to 1.5 —plants that are given monthly treatments of 0.2, 0.4, 0.6 and 0.8 g. respectively, of ammonium nitrate.

FIG. 2   *Perestrophe bicalyculata* Nees, plants growing in partial shade in soils treated with different doses of ammonium nitrate.
    FIG. 2.1 —control; FIGS. 2.2 to 2.5 —plants that are given monthly treatments of 0.2, 0.4, 0.6 and 0.8 g. respectively, of ammonium nitrate.

FIG. 3   *Perestropha bicalyculata* Nees, plants growing in deep shade in soils treated with different doses of ammonium nitrate.
    FIG. 3.1 —control; FIGS. 3.2 to 3.5 —plants that are given monthly treatments of 0.2, 0.4, 0.6 and 0.8 g. respectively, of ammonium nitrate.

appears to be more important than the genetical potential. On the other hand, we have in *Euphorbia thymifolia* Lin. (Ramakrishnan 1959) two eco-types—the red form and the green form, the former of which is able to thrive in calcareous as well as non-calcareous substratum. The green form thrives only in non-calcareous soils and is able to utilize calcium from only such soils.

SUMMARY

(1) This paper deals with the ecology of *Peristrophe bicalyculata* Nees.

(2) *P. bicalyculata* Nees. occurs in nitrogen poor soil in the open and nitrogen rich soil in partial shade. This has been explained as due to favourable germination and growth of the plants under these conditions.

(3) A study of plant nitrogen in relation to soil nitrate shows that, in general, the plants in partial shade are richer in total nitrogen. With increase in nitrate in the soil, a decrease in total plant nitrogen has been recognized; the reverse being true in partial shade and deep shade. In deep shade, the nitrogen content of the plant is very high which along with low light intensity results in very poor growth of the seedlings.

(4) The average seed output of the plant is lower in partial shade than in the open.

(5) Seedling regeneration is at its best in partial shade. In deep shade seedlings die away soon.

(6) The reproductive capacity is higher in the open than in partial shade, but the value for aggressive capacity works out to be the same under both conditions due to a higher percentage survival of seedlings in partial shade.

(7) The stomatal frequency of 'sun' and 'shade' leaves and the range of osmotic pressure of the plant sap are given.

(8) Dispersal of the seeds is by an explosive mechanism and its effectiveness has been sketched. Seedling morphology is also described.

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A STUDY OF SANTAL FAMILY SIZE

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During 1945-46, a survey was made of Santal villages in Bengal by the present writer. A complete list of all Santal villages in the six subdivisions of Bankura Sadar, Jhargram (in Midnapur district), Midnapur Sadar, Birbhum Sadar, Maldah and Balurghat (in Dinajpur district) where Santals live in large numbers was obtained from the Government of Bengal (then undivided). Pilot surveys of an intensive kind had been carried out by the writer earlier in certain Santal areas spread over a period of several years at intervals. On the basis of the data so collected, it was considered advisable to draw separate random samples for each subdivision, i.e. to resort to stratified random sampling. A schedule was prepared by the writer on the basis of the data collected in the Pilot survey and the questionnaire was also standardized to be of 'simple' type, i.e. capable of being answered only by a yes or no, a name or a number or one specific reply out of several definite alternatives. In all, nearly eleven hundred families in seventy-one villages were studied. For every family, a short genealogy was collected and various economic and social data obtained by using the genealogical method of enquiry. A report designed to help administrators was drawn up and submitted to Government within a year of completion of the survey (Chattopadhyay 1947). Other data are being gradually published. In this paper a study will be made of the number of children in the different age groups of married women in the Santal families in two subdivisions out of those surveyed, to ascertain whether greater spread of literacy has had any effect on family size among them.

A comparison of the number of children per married woman can be validly made for different areas, only in those cases where the age group distribution of married women is similar. For such a comparison the two subdivisions of Maldah and Jhargram are suitable, on this ground. The number of married women are shown in somewhat broad age groups, in Table I, for these two areas.

It is evident that the proportion of married women out of the total number studied (excluding those whose age was not stated) is about the same in the three broad age groups noted. This division has been made as roughly about half are in the highest age group, and the rest are in the two lower groups. The number of children for the married women of these areas is given in Table II below.

TABLE I

Number of married women by age groups

Area	Age up to 20 years	Age 21 to 30	Age 31 and above	Total
Maldah	43	64	79	186
Jhargram	43	67	82	192

TABLE II

Number of children by age of the married women

Area	Age up to 20 years	Age 21 to 30	Age 31 and above	Total
Maldah	32	128	274	434
Jhargram	27	125	290	442

Again, in any comparison to ascertain the effect of any particular variable, other things have to be kept constant.

In 1943 a famine of terrible intensity swept over the greater part of Bengal. Some subdivisions were badly affected, others moderately so, and some were only lightly touched by it. In the rest of Bengal food scarcity and high prices prevailed, but not to the extent described officially as a famine. Among these areas, both Maldah and Jhargram escaped the serious grip of the famine.[1] The economic condition of the Santals was also similar in both the areas.

Most of the Santals in these two subdivisions make their living by cultivating land, part of which is owned by them (as tenants) and part of it is taken over on sharecropping basis. As half the crop, in the case of sharecropping, is made over (used to be in 1945-46), the area of such land may at least be halved to estimate the effective amount of land cultivated by a family. The following table gives the average acreage for owned, as also for owned plus half of what is sharecropped by a family, and also the distribution by acreage within the group. A small proportion of families owning a big acreage of holdings may unduly inflate the average and convey a wrong picture of the economic condition. Hence the distribution by acreage is noted.

It may be noted here that adding half the acreage of crop-shared land leads to a slightly higher estimate of area of holdings than is justified, as the cost of cultivation is not less than twenty-five per cent of the produce and

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[1] A sample survey of the famine-affected areas all over Bengal was carried out by the writer in 1944-45, with the technical help of Prof. P. C. Mahalanobis and with financial help of the Government of Bengal. The reports have been published by the Indian Statistical Institute.

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TABLE III

Distribution by per cent of families

Area	Actual number of families	Average acreage per family	Distribution per cent		Over 5 acres
			0–2 acres	2+ to 5 acres	
Jhargram, owned land	145	2·7	49·7	35·9	14·4
Jhargram, owned plus half-shared	145	3·3	35·9	46·9	17·2
Maldah, owned land	138	2·3 acres	60·9	27·5	11·6
Maldah, owned plus half-shared	138	3·7 acres	31·1	48·6	20·3

has to be borne by the peasant tilling the land, and not by the owner who gets half the crop harvested.

In the above distribution of acreage, therefore, the somewhat better position of Maldah Santals suggested by the acreage held, after inclusion of half the land shared, is not very significant. If only two-fifths of the area of land taken on a sharing basis are included as more in accord with reality, the apparent advantage of Maldah Santals disappears. On the other hand, there is always the uncertainty of being able to obtain enough land on a sharing basis. The economic security felt through ownership (settled tenancy) of land is of importance in its effect on standard of life and, in general, on the way of life. On the whole, therefore, it may be said that Jhargram Santals are not worse off than Maldah Santals, so far as income from land is concerned. It will be clear, however, that in both cases less than one-fifth of the total number of families (considering owned land plus two-fifths of share land) has economic holdings. Of the rest about a third can make their living from land; the rest do not have even this little bit of land. The following table of occupational percentages for the two subdivisions throws further light on this point. By 'Agriculture, and Agriculture and Labour' is meant persons who only cultivate their own land and those who so cultivate and else engage in work as labourers a part of the year. The category 'Agricultural Labour' includes those who live practically by work as labourers only.

The Maldah Santals are thus entirely dependent on work in the fields, their own, or of others. In Jhargram, on the other hand, there were several families who earned money by teaching, while two families had a son each in the Pioneer force. Literacy in the area of Jhargram surveyed was quite high being 21·8 per cent of the population above five years of age. It may be noted here that this high percentage of literacy does not apply to the whole subdivision but only to the area surveyed as one village in the first sample

TABLE IV

The occupational percentages

Area	Agriculture, and agriculture and labour	Agricultural labour	Service	Total
Jhargram Sadar	82·1	13·8	4·1	100·0
Maldah	90·6	9·4	nil	100·0

was in Thana Gopiballavpore and was not visited by the investigators as being difficult of access in the season of the survey. In its place, another village from Thana Binpore was included. While this gives extra weight to this thana, it does not affect the validity of comparisons made in this paper, so long as it is remembered as being restricted to the area surveyed. Also, in the sample itself, except for literacy, there were no appreciable differences in economic conditions and social customs in the thana left out and those included.

It is clear from the figures in Table I and Table II that Maldah and Jhargram Santals have about the same number of children per married women in the different age groups. As the age groups of married women correspond and the economic conditions are closely similar, it may be concluded that the much higher literacy of the Jhargram Santals has had no effect on the number of children born to them.

It would, however, be a mistake to conclude that the increased literacy has had no effect on the Santals in Jhargram in other respects.

It was noted in the report on the survey already mentioned that the Santals in Jhargram had in the Binpore Police Station a well-organized Dharmagola which had arranged for the entire requirement in the matter of grain loan of the villagers of the place where it had been set up. It showed an awareness of the need of such co-operative effort by themselves to escape the grip of the Mahajan. Among Santals co-operation in hunting and fishing and looking after the needs of villagers is traditional. It would appear that the high degree of literacy in the particular area led them to utilize that tradition of co-operation in the modern field of a Dharmagola of which they had seen models among some of their neighbours.

It may also be noted, that the Santal tends to improve his standard of living with better economic conditions apart from increase in literacy. This is evidenced, for example, by data from house types. A Santal normally needs a dwelling with two huts. A married son does not at once set up a separate hut for himself. In the house one room has at one end the sacred place for ancestors; it may also be the *daka orak* or kitchen. The other would be a simple living-room. In the survey mentioned it was found that Santal

families had dwellings ranging from one hut to seven huts. It was found that if the family is large and resources allow it, more rooms are built beyond the two usually needed. A poor man, on the other hand, had to combine in one hut the functions of the shelter for ancestors, for the kitchen and for sleeping. A tabulation of dwelling size on the basis of number of rooms and the amount of land cultivated (owned plus half of shared) shows the correlation between the two sets of data. The acreage has been shown as 0–5 acres and 5+ acres, in view of the fact that five acres is an economic holding and allows comfortable living for a family. The figures given are percentages of the total number.

TABLE V

Dwelling size on the basis of number of rooms, and the amount of land cultivated

Area	Acreage held		Families with	
	0 – 5	5+	Two huts	More than two
Jhargram	82·8	17·2	77·9	22·1
Maldah	79·7	20·3	67·4	32·6
Midnapur Sadar	98·1	1·9	93·7	6·3

The correlation holds also for the other subdivisions. As houses are built in one generation and last into the next, and as land sales or transfers had occurred due to the worsening economic situation in general from that of the generation prior to that in charge in 1945-46, the lag in the percentage of number of huts behind that for land is not unexpected. But the evidence is unmistakable that Santals, when better off, try to live more comfortably. In this respect they try to follow their neighbours, the better-off Hindu peasantry, whom they admire in spite of the dislike expressed by the term 'Diku' for that element among Hindus for whom they have a hostile feeling. In another publication it has been pointed out by the writer (Chattopadhyay 1959) that the Santals in Midnapore as well as Jhargram subdivision under pressure of economic conditions and other forces have had changes in certain social traits. It has been shown that a particular institution like marriage, of which there are expensive as well inexpensive forms, as also informal types with later formalization, may in adjustment to economic changes have the relative frequency of different types altered. In areas where there are few respected outsiders, the major effective force may be of economic change. But where external forces arising from continuous contact with a respected outside culture are operative, the direction of changes is only in part determined by economic forces. The indirect social force developed by the desire to conform to the way of life of the respected outsiders, provided such conformity

does not go against the integrated earlier culture, is in this case quite important as a determinant of change. In Jhargram, where the Santals have better preserved their earlier culture, the social force arising from external contact has been less effective; in Midnapore, where there has been a good deal of disintegration of the earlier culture such social forces have been more effective in determining changes. In both cases the effect of the influence of Hindu society is apparent.

It is a feature of Hindu society in the villages, that no attempt is made at birth-control. A recent enquiry in a rural area in Midnapore by a research scholar (Chattopadhyay, G. (unpublished)) attached to this department showed that very few Hindu residents of the villages are aware of modern methods of contraception; the few, who have knowledge of it, do not practise birth-control. All the local Santals were found to be ignorant of such methods. There are reports elsewhere of their own way of trying to secure non-fertilization in pre-marital relations. But no authentic information or evidence is available. It is evident that the imitation of Hindus in certain matters has not helped the Santals towards family planning on modern methods.

In a paper published earlier (Chattopadhyay 1957) it has been pointed out that the major objective of polyandry among Todas, Tibetans, Ceylonese and Khasas of Jaunsar Bawar was the retention of a definite standard of economic security. In the Marquesas Islands the prestige motive was a close parallel. The Nambudiris of Kerala area allow only the eldest son to marry to keep the property in one family from generation to generation. Polyandry of the type practised by the people noted has the same effect. In modern times, the limitation of offspring among higher income groups in England and United States of America in the last century illustrates the operation of the same socio-economic force. The procedure followed to attain the same end is, however, different.

It has been noted earlier in this paper that the Santal adopts a more comfortable way of life when means permit. Awareness of desirability of better standard of life is therefore present. He attempts also to adjust his customs in a changed social and economic environment. Earlier traditional practices are also being reinterpreted in modern terms by them where suitable models are observed by them among neighbours. The Santal community has therefore shown itself not to be blindly conservative, and has displayed capacity for initiative. At the same time it is clear that family planning has not any hold in rural areas either among their more advanced neighbours or among them. The desire to assure economic security to descendants which has operated among other simpler folk as well as advanced people, as indicated in the previous paragraph, has not apparently led the Santals in the direction of family planning. The requisite knowledge is not available to them; but something more is lacking. It is not a practice that is followed among people whom

they openly or secretly look up to; also the economic condition of the majority of Santals is not such that they can think of economic security when living at that level. Improvement of economic condition is essential if such forces are to arise, apart from other factors. Spread of literacy is not by itself adequate.

The study of a comparatively simple folk has been made to ascertain how various forces, that may affect family planning, operate among them. It seems likely that some of the conclusions apply also to our rural folk, to spread the practice of family planning among whom very large sums of money are being spent by our Government. Undoubtedly the forces operating in our rural society are more complex. There are also class divisions. Some of the factors operating in such societies have been discussed in the paper on study of social changes already referred to earlier in this paper. It is desirable that certain essential conditions that encourage the rise of forces in favour of family planning should be taken into account when launching on an expensive programme for its adoption by our rural folk.

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THE PROBLEM OF RHIZOSPHERE MICROFLORAS

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The rhizosphere was defined by Hiltner (1904) as a soil ecological group inside which the soil is subject to specific influence of plant roots. Ever since then many workers have investigated this rhizosphere 'effect' in an attempt to study the many interactions that take place between the several groups of microorganisms of this 'metabolically active' region. In fact, root surfaces are a more potent source of energy than the soil adjacent to roots and this region is termed the 'rhizoplane' which includes external surfaces of plant roots and closely adhering soil or debris. Interest, therefore, has now shifted to a study of the exudates of plant roots and the neat and precise modern analytical tool, chromatography, has enabled deep penetration and understanding of this region of activity and aided in elucidating some aspects of the complexity of this symbiosis. It is well known that, in soils, autotrophic and heterotrophic microorganisms occur as mixed populations. While the autotrophic microorganisms synthesize their energy requirements from simple inorganic substrates, the facultative and obligate heterotrophic forms require preformed carbohydrates, proteins, peptone, amino acids and vitamins for growth. It is precisely this group of microorganisms that occurs in large numbers in the rhizosphere and constitutes one of the most interesting problems for study especially in the field of root exudates, their quality and quantity and their effect on this microflora. With increasing knowledge of the micropopulation of these rhizosphere regions it may even be possible, in course of time, to explore the possibility of the occurrence of 'ecospecies' among the microfloras in the rhizosphere of many crop plants, which seem to exist in that environment, judged from results obtained here and elsewhere, but at present lacks experimental proof.

These exuded metabolites from plant roots represent a wide range of substances like amino acids, sugars, nucleotides, flavanones, etc. Possibly other substances, hitherto unidentified, may be present and await exploration. However, we have evidence to show that in the region of the root changes can be induced so as to produce possibly new exudation complexes or specific mutual antagonisms between the microorganisms by aiding the production of specific inhibitor(s) of microbial metabolism by the plant or the selective production of antibiotics inimical to the growth of one another. One such evidence is the effect that seems to be induced by foliar sprays of urea on

Oryza sativa L. when a change in the rhizosphere floras occurs in the sprayed plants as compared with the unsprayed controls (Ramachandra-Reddy 1959). Two to five sprayings of 0·1 M solution of urea with a detergent on the foliage of a strain of rice plant susceptible to the foot-rot disease caused by *Fusarium moniliforme* Sheld. gave in the rhizosphere a higher fungal count and a lower bacterial and actinomycete number as compared with the unsprayed plant in the same soil aliquot.   Apart from this quantitative effect on the microflora of the rhizosphere, urea sprays seem to promote selectively the growth of a particular species of *Penicillium*.   This was explained by putting forward two hypotheses: (*a*) preferential stimulation in the rhizosphere of *Penicillium* sp. and the consequent reduction of actinomycete and bacterial numbers in the urea sprayed plants and (*b*) inhibition by the possible production *in situ* of antibiotics by the *Penicillium* group.   A somewhat similar approach to the study of rhizosphere microfloras of one-year-old monoclonal selection of tea plants (*Camellia sinensis*) was made by Venkata Ram (C. S. Venkata Ram, personal communication).   His results seem to indicate, among other observations, a general concurrence with the results of Ramachandra-Reddy in that foliar spray of potassium chloride, magnesium sulphate and nitrogenous substances, organic and inorganic, did bring about a basic change in the pattern of the rhizosphere microflora, although on the specific effect of urea there was not much parallel but the plant chosen for his study was a dicot and the soils and other environmental conditions totally different to that of Ramachandra-Reddy.   Venkata Ram's results also indicate that among the various nitrogenous compounds sprayed containing comparable amounts of N, Na_2HPO_4 and KCl significantly increased the number of fungi in the rhizosphere soil over that in the control, whereas a significant reduction of fungal numbers was recorded with application of $MgSO_4$.   The point of further interest emerging from this study was that more genera and species of fungi appeared in the rhizospheres of plants receiving different sprays over unsprayed plants growing in similar soils.   This would indicate the importance of the concept of foliar spray in studying rhizosphere microfloras in general and in the isolation of more genera and species which normally do not show up in soil dilution plates.   While it is known that roots of plants subjected to high temperature or light intensity (Rovira 1959) or plants grown under desiccated soil moisture conditions (Katznelson *et al.* 1954) exude large amounts of amino acids, such specific effects on the quality and quantity of the soil microfloras have not been so effectively demonstrated as in this foliar spray concept and rhizosphere floral changes.

Indeed, study of root exudates has assumed a new importance especially in understanding pathogenesis and in the production of antibiotics and toxins by soil organisms in the rhizoplane and in the rhizosphere.   One of the notable contributions in this field is the detection of fusaric acid (5-n-butyl pyridine

carboxylic acid) in sterilized soils given organic amendments (Kalyanasun-
daram 1955). As much as 7·9 μg./g. of this antibiotic was formed by *Fus-
arium vasinfectum* in sterilized soils amended with green leaf and oats. This
idea was further extended to a study of production of fusaric acid in the rhi-
zosphere using *Fusarium lycopersici* cultures inoculated to growing tomato plants
in sterilized soils and sand cultures (Kalyanasundaram 1958). The exudates
and metabolites when examined showed in the rhizosphere of inoculated sand
as well as soils about 7·5 μg. and 11·25 μg. fusaric acid on an average in the
rhizosphere of a single 20-day-old tomato plant, 48 hours after inoculation of
the soil with the pathogen. It would appear, therefore, that for the free
growth in the rhizoplane and for successful production of this antibiotic the
fungus depended largely, if not solely, in the sand cultures at least, on the root
exudates of these tomato seedlings. It is obvious that further work on these
lines using isotope techniques for more critical evaluation of the production of
antibiotics *in situ* would be very rewarding. Further, the specific metabolic
changes that foliar sprays of substances like sugars, amino acids and other
nitrogenous compounds as well as inhibitors such as azaguanine, thiouracil,
etc., and accelerators of metabolism like kinetin bring about in the foliage and
in the root exudation pattern have to be studied intensively.

The effects of root exudates *in vitro* on physiologic races of *Fusarium
oxysporum* Fr. *f. pisi* have been studied recently (Buxton 1957a, b). Root
exudates from three pea cultivars capable of differentiating between three
physiologic races of *F. oxysporum f. pisi* affected spore germination in the
three races, depressing the germination of races they resisted more than those
to which they showed susceptibility. Further, exudates from roots of young
pea seedlings depressed germination more than exudates from older plants.
Bhuvaneswari (1958) tried the effect of exudates of resistant and susceptible
rice varieties on spore germination and growth of the foot-rot fungus *Fus-
arium moniliforme* Sheld. *in vitro* and reported inhibition with the exudates
from resistant rice and more favourable growth conditions with exudates from
susceptible plant roots as compared with water control (Table I). This obser-
vation had further experimental proof as *F. moniliforme* showed its ability to
survive in the rhizospheres of the susceptible plant which was twenty-fold
greater than in that of the resistant rice variety. Studying the effects of the
same pea cultivars Buxton (*loc. cit.*) showed that they exerted different effects
on the soil microflora. The cultivar susceptible to *F. oxysporum f. pisi* race 1
supported near its root surface more fungi, bacteria and actinomycetes than
either of the cultivars which resist race 1. Spores of a race capable of wilting
a particular cultivar germinate well in soil extract from rhizosphere of that
cultivar and, on the other hand, germination is decreased in extracts from the
rhizosphere of a resistant cultivar. These results suggest that substances
exuded by roots of cultivars resistant to race 1 alter the soil microflora,

TABLE I

Effect of root exudates of resistant and susceptible rice varieties on growth of Fusarium moniliforme

Treatments	% Spore germination (in water)	Length of germ tube in μ in water	Radial growth in cm. Czapek's agar	Dry wt. in mg. Richard's medium
Control ..	90	10·8	4·3	7·7
Exudate from resistant plant ..	23	3·6	1·1	negligible
Exudate from susceptible plant..	92	16·8	9·2	9·6

preventing race 1 from germinating and thereby lowering effective inoculum level and delaying wilting.

A somewhat similar approach was made earlier by Agnihothrudu (1954) who showed that the rhizosphere of the wilt-susceptible pigeon pea variety (*Cajanus cajan* (Spreg.) Millsp.) was more suited for the survival of the causal agent *Fusarium udum* than were the rhizosphere of the resistant varieties tested. Also a sizable actinomycete population antagonistic to *F. udum* was present in the rhizosphere of resistant plants. It appears, therefore, that sharply defined genetic varieties (as wilt resistant or wilt susceptible) of crop plants belonging to many genera and species should be screened in large numbers for their quality and quantity of root exudates under controlled environment. Also, their rhizosphere floras should be critically studied if we were to understand this exudation problem and its many sided influences on the microbial habitat of the rhizosphere.

In this laboratory, Sulochana (1958) has studied, using bioassay techniques, root exudates of two genetic strains of cotton and correlated it with the microfloras of the rhizosphere. The cotton chosen were several strains of diploid *Gossypium arboreum* race *indicum* L. susceptible to *Fusarium vasinfectum* and amphidiploid resistant *G. hirsutum* L. Quantitative assays for amino acids using the test organisms *Lactobacillus arabinosus* 17/5 and *Leuconostoc mesenteroides* P. 60 were made and similarly vitamin B group assays were done using X-ray mutants of *Neurospora crassa* and *N. sitophila*. The results are presented in Tables II and III.

The results (Table II) indicate that qualitatively there seems little difference in the pattern of distribution of amino acids in the various healthy diploid and amphidiploid strains except that cystine, which is considered to be a resistance factor, seems to be exuded more in the diploids than in the amphidiploids. However, quantitatively, taking into account all the amino acids recorded and estimated, the healthy diploid susceptible plants seem to have a much higher quantity of amino acids as compared with the amphidiploids.

TABLE II

Amino acids in root exudates of diploid, susceptible and amphidiploid resistant strains of cotton (Microbiological assay: Organisms—Lactobacillus arabinosus *17/5 and* Leuconostoc mesenteroides *P. 60*)

Amino acid	Unplanted control soil	Diploid susceptible strains of G. arboreum race indicum L.			Amphidiploid resistant strains of G. hirsutum L. Co2 MCU1 MA5 Laxmi			
		K2	K5	6186·9	Co2	MCU1	MA5	Laxmi
	microgram in 250 ml.							
Threonine	0	0	0	0	0	300	0	0
Glycine	195	740	610	390	430	510	640	330
Leucine	0	270	280	410	370	380	420	350
Phenylalanine	0	1100*	400	600	300	600	0	750
Aspartic acid	0	1200*	0	1200	0	1300	600	0
Lysine	320	1480	1440	1440	640	320*	800	560*
Histidine	0	1200	1150	700	750	1500	0	650
Serine	1110	2400	1650	1910	1380	1920	1570	1930
Proline	1200	2240	1860	2200	1390	1880	1620	1880
Arginine	108	130	160	135	168	220	165	140
Cystine	0	345*	580*	576*	0	535	0	0
Total :								
Healthy	2933	11105	8130	10551	5428	9465	5815	6590
Inoculated	2933	5498	5930	4295	**	6625	6345	4075

* Absent in the inoculated series.
** Not assayed.

The inoculated wilting susceptible plants when examined showed a considerable diminution in the total amino acids exuded, or perhaps it would be more accurate to call it total amino acids detected. Obviously, in the presence of the inoculum it would be difficult to assess quantitatively whether the deficit in the rhizosphere was due to decreased synthesis by the host during pathogenesis or a decreased detectable quantity in the rhizosphere consequent on the depletion of these readily assimilable forms of nitrogen by the pathogen in the rhizosphere as, indeed, we have evidence to show that pathogenic fungi can grow and thrive in the rhizosphere with little recourse to external sources of food material. Be that as it may, Sulochana's results seem to indicate that in the total amino acid exudation pattern there are basic differences between the diploid and amphidiploid strains of cotton examined and, on pathogenesis, the quanta recorded for the susceptible diploid strains go down. To this extent it is probably one of the first evidences to indicate that it appears to be a gene controlled phenomenon worthy of more elaborate experimentation. As regards vitamin exudates, even here the diploid plants exude more than the amphidiploids and that on pathogenesis there is considerable

TABLE III

Vitamins in root exudates of diploid, susceptible and amphidiploid resistant strains of cotton
(Microbiological assay : organisms—X-ray mutants of Neurospora crassa *and* N. sitophila*)*

Vitamins	Unplanted control soil	Diploid susceptible strains of *G. arboreum* race *indicum* L.			Amphidiploid resistant strains of *G. hirsutum* L.			
		K2	K5	6186·9	Co2	MCU1	MA5	Laxmi
		microgram in 250 ml.						
Thiamine ..	0	16·25	2·50	25·00	1·68	2·00	2·36	2·00
Biotin ..	Trace	8·75	7·50	8·89	2·00	8·12	5·25	3·75
Pyridoxine ..	8·00	13·00	15·00	13·50	10·50	13·20	8·70	9·50
p-amino benzoic acid ..	0·25	3·51	1·53	7·55	1·48	1·75	0·85	1·06
Total :								
Healthy ..	8·25	41·51	26·53	54·94	15·66	25·07	17·16	16·31
Inoculated..	8·25	15·18	17·28	16·88	**	19·25	15·13	11·60

** Not assayed.

diminution in the diploids although the amphidiploids also show some slight fall in the amounts exuded (Table III).   One fact emerges from these investigations and that is, the need to study plasma membranes of root systems of genetic varieties for their semi-permeability qualities and also under controlled environments and varying nyctotemperatures exudation patterns of amino acids, organic acids, sugars, vitamins, etc.

We have recently studied rather intensively root exudates of the rice plants, resistant and susceptible to the *Fusarium moniliforme* foot-rot disease (Bhuvaneswari, *loc. cit.*).   The susceptible rice plant exuded greater quantities of amino acids (except cystine) and sugars but comparatively less of organic acids.   The resistant plant, on the other hand, exuded sizable quantities of cystine, which appears to be a resistant factor, and at least three organic acids, of which citric acid appears to be higher than oxalic and tartaric acids recorded (Table IV).   A somewhat analogous situation exists in the exudate pattern of organic acids in *Brassica juncea*.   Here again considerable quantities of malic and citric acids were exuded.   These organic acids of the rhizosphere of *B. juncea* determined chromatographically were eluted and tested against the natural soil flora in agar Petri plates.   Citric and malic acids decreased the bacterial numbers of both the control and rhizosphere soils. This decrease in bacterial numbers was observed when the citric or malic acids were added either singly or in combination with the other acids.   However, the depressing effect of citric acid was found to be greater than that of

TABLE IV

Root exudate pattern of rice varieties resistant and susceptible to foot-rot

Amino acids			Resistant variety	Susceptible variety
Cystine	..	..	+ + +	—
Aspartic acid	..	..	+	+ + +
Asparagine	..	..	+	+ + +
Glutamic acid	..	..	+	+ +
Tryptophan	..	..	—	+ +
Methionine	..	..	—	+ +
Valine	..	..	+	+ +
Nor-leucine	..	..	+	+ +
Sugars				
Maltose	..	..	—	+ + +
d-Glucose	..	..	+	+ + +
d-Fructose	..	..	+	+ + +
d-Xylose	..	..	—	+
Unidentified	..	..	—	+
Organic acids				
Oxalic	..	..	+	+
Tartaric	..	..	+	—
Citric	..	..	+ + +	—

malic acid. Despite the fact that exudation of organic acids from roots seems to be a general feature of the many crop plants analysed, its full import on rhizosphere floras, saprophytic or pathogenic, has to be further carefully examined, although admittedly two of the organic acids, malic and citric, have a depressing effect *in vitro* on bacterial numbers.

The disease syndrome of foot-rot of rice comprises of two distinct symptoms, the 'bakanae' symptom or the internodal elongation (a gibberellin effect) and the typical foot-rot diseased condition. Bhuvaneswari (*loc. cit.*) has studied the physiological groups of soil bacteria in the rhizosphere of resistant and susceptible rice plants and also under 'bakanae' and foot-rot conditions (Tables V–VII). It is apparent that the healthy susceptible variety recorded greater numbers of physiologically active groups of bacteria than the resistant variety (Table V). However, among the nutritional groups of bacteria present in the rhizosphere the healthy susceptible variety had in its rhizosphere a greater percentage of organisms requiring amino acids (Group II) than organisms requiring amino acids plus growth factors (Group III) as compared to the resistant variety (Table VII). The resistant variety, however, appeared to stimulate a greater percentage occurrence of organisms requiring simple substances present in the basal medium as compared to that of the healthy susceptible variety (Group I). Foot-rot and 'bakanae' plants, however, showed a different picture. In general, foot-rot plants stimulated a

TABLE V

Percentage occurrence of physiological groups of bacteria in rhizospheres of rice varieties resistant and susceptible to foot-rot

Physiological groups	Resistant	Susceptible
Nitrifying	17	43
Denitrifying	10	19
Cellulose decomposing	7	20
Gelatin liquefying	15	18
Starch hydrolysing	9	33
Azotobacter	1	1
Ammonifying	13	23

TABLE VI

Percentage occurrence of physiological groups of bacteria in rhizospheres of rice plants showing foot-rot symptoms and 'bakanae' effect

Physiological groups	Foot-rot symptoms	'Bakanae' effect
Nitrifying	14	20
Denitrifying	27	9
Cellulose decomposing.. ..	32	10
Gelatin liquefying	35	5
Starch hydrolysing	38	3
Azotobacter	0	1
Ammonifying	13	23

TABLE VII

Percentage occurrence of nutritional groups of bacteria in rhizospheres of rice varieties, resistant and susceptible to foot-rot

Media	Resistant	Susceptible
Group I. Basal	70	10
,, II. Basal + vitamin free casein hydrolysate	24	60
,, III. Group II + growth factors ..	4	24
,, IV. Basal + yeast extract ..	2	4
,, V. Basal + soil extract	0	2
,, VI. Basal + yeast and soil extracts ..	0	0

greater percentage occurrence of physiologically active forms, particularly in groups III, IV and V than those showing 'bakanae' symptoms (Table VI). One point is obvious: plants with foot-rot symptoms appear to exude a larger quantity of energy substances but not perhaps a variety of energy materials

capable of supporting a larger number of bacteria in the seven physiologically active groups investigated than plants showing obvious 'bakanae' symptoms. This concept of studying the metabolism of foot-rot and 'bakanae' plants using the rhizosphere floras as an index to their physiological grouping is a good one and needs more elaborate experimentation.

Notwithstanding the numerous studies made in the field of relation of underground plant parts to environment, the importance of root exudates on the soil microfloras has not attracted sufficient attention until very recently, although familiar illustrations of symbiosis such as the mycorrhiza and nodule bacteria are much quoted examples. Many new interactions between soil microorganisms and plant roots are being described in increasing measure and this has undoubtedly stimulated new thinking. It seems obvious that there is an urgent need to evaluate the nature and quantity of various exudates (both energy substances and inhibitors of metabolism) likely to occur not only in roots but also in leaves of crop plants under controlled environment. It is equally important to work on physiologic races of soil-borne fungi and gene-controlled mechanisms governing root exudations of genetic plant varieties that bring about physiologic groupings of bacteria in the rhizosphere of these plants. The nature and extent of production of antibiotics in the rhizosphere *in situ* by microorganisms under aseptic conditions of growth have yet to be studied. Indeed, techniques that would aid such evaluation have to be evolved. There is, thus, a vast and almost maiden field open to the physiologist and pathologist for collaborative research into these newer concepts of the problems of the rhizosphere microfloras and their effects on host metabolism and *vice versa*.

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STUDIES IN THE DISEASE OF *MANGIFERA INDICA* LINN.

XII. FURTHER STUDIES IN THE EFFECT OF BORON ON MANGO NECROSIS

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ABSTRACT

Field experiments were conducted in two different heavily necrotic orchards to find out the effectiveness of boron on mango necrosis by spraying the trees with borax solution at each stage of preflowering, during flowering and early fruiting, setting aside an adequate number of untreated trees as controls. The statistical analysis of the results by Chi-square test of independence clearly goes to show the effectiveness of boron in preventing necrosis, thus confirming the results of the previous experiments. It is suggested that boron applied in adequate doses and at appropriate time is a good practicable method for controlling necrosis.

INTRODUCTION

The necrosis of mango fruit, also designated as blacktip because of the characteristic final symptom of the disease, has long been known to be occurring in orchards situated near operating brick-kilns (Das-Gupta and Verma 1939; Pal *et al.* 1937; Sen 1941).

Nutritive and trace element experiments to control the disease gave some indication that nutritional deficiency is in some way responsible for the development of necrosis (Das-Gupta 1951). Thus branch injection of Knop's solution appeared to check the disease at its early stages. Later and more recent experiments had also shown that adequate application of boron in the form of spray checks or prevents necrosis depending upon the time of application. When boron is sprayed after mangoes have developed early symptoms the further progress of the disease is checked while the disease is significantly prevented when the spraying is done well before its incidence (Das-Gupta and Sen 1958, 1960). The corroboration of these was sought in a further intensive investigation on the effect of boron sprayed on trees known to be affected with mango necrosis the results of which are presented in this paper. The main conclusions have already been communicated in note form (Das-Gupta and Sen 1959).

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TABLE I

Showing details regarding orchards and their relation to brick-kilns

ORD. I, Bahadurpur.   ORD. II, Panigaon

Orchard	Plot	Number of trees in each plot			Operating brick-kilns			Wind direction	Remarks
		Trees fruiting	Trees not fruiting	Total number of trees	Total number	Direction	Distance from the centre of the orchard (in furlongs)		
ORD. I	A	60	39	99	5	1—West 1-4—North-west (close together)	1¼ 4	Mostly towards east. Sometimes towards west.	Plot A is nearest to the brick-kilns and plot D farthest from it.
	B	123	33	156					
	C	94	153	247					
	D	106	206	312					
	Total	383	431	814					
ORD. II	A	19	64	83	3	1—West 1—South 1—South-east	1 3 4	Mostly towards east. Sometimes towards west.	
	B	8	21	29					
	C	42	100	142					
	D	11	41	52					
	E	12	75	87					
	Total	92	301	393					

MATERIAL AND METHODS

Orchards.—Two different orchards (ORD. I and ORD. II) in the neighbourhood of Lucknow composed almost exclusively of trees belonging to the varieties of *dasheri*, *safeda* and *khajri*, highly susceptible to necrosis and known to suffer regularly from heavy incidence of the disease, were utilized for spray experiments. ORD. I situated in Bahadurpur comprised four plots A, B, C and D. This orchard had five operating brick-kilns in its vicinity —one on west and four others in a north-westerly direction. The plots A and B were generally more affected than plots C and D. ORD. II situated in Panigaon about five miles from Bahadurpur had three operating brick-kilns—one on west and two others in a south-west direction. This orchard consisted of five plots A, B, C, D and E of which A and B are towards west and C, D and E towards east of an irrigation canal. The details regarding the two orchards and their location in relation to brick-kilns, the number of trees bearing fruits that season and other relevant data are given in Table 1.

Spray.—The spray consists of 6 lb. of Twenty Team Mule borax in 100 gallons of well water in ORD. I and the same amount of borax dissolved in 100 gallons of canal water in ORD. II. The spraying was done by means of a power sprayer operating at 200 lb. pressure. The trees were sprayed both from inside and outside till all the leaves were thoroughly wetted resulting in slight dripping (Das-Gupta and Sen 1960).

The plots A, B and C of ORD. I and plots A, B, C and D of ORD. II were sprayed thrice during the year before flowering, during flowering and immediately after fruit setting as indicated in Table 2. The trees in plot D in ORD. I and plot E in ORD. II were not treated and served as controls.

TABLE 2

Showing the bearing condition of trees and the dates of spraying

Orchard	Plot	Spray water used	Spraying date		
			Preflowering	During flowering	Early fruiting
ORD. I	A	Well water	2-2-58	7-4-58	3-5-58
	B	,,	2-2-58	8-4-58	4-5-58
	C	,,	9-2-58	5-4-58	1-5-58
	D	Unsprayed control			
ORD. II	A	Canal water	7-2-58	2-4-58	28-4-58
	B	,,	7-2-58	2-4-58	28-4-58
	C	,,	6-2-58	3-4-58	29-4-58
	D	,,	6-2-58	3-4-58	29-4-58
	E	Unsprayed control			

The trees were kept under careful observation to note the progress of fruiting and the incidence of necrosis.

A final fruit count was made when the majority of fruits had attained a size of four inches and the number of healthy mangoes and those showing different stages of necrosis were recorded.

RESULTS

The results obtained from experiments performed in ORD. I and ORD. II are given in Tables 3 and 4 respectively. It will be observed from Table 3 that among the treated trees out of a total of 15,107 fruits only 195 were necrotic (1·28 per cent) while among the controls 1,976 out of 3,046 fruits became necrotic (64·87 per cent). Table 4 shows that among the treated plants out of a total of 2,211 fruits only 29 were necrotic, the percentage of necrosis being 1·31; among the controls 158 out of 362 fruits became necrotic, the percentage of necrosis being 43·65.

TABLE 3

Showing the amount of necrosis in treated and control plants in ORD. I

Plot	Treated or Control (T or C)	Number of healthy fruits	Number of diseased fruits	Total number of fruits	Percentage of necrosis
A	T	1,622	60	1,682	3·57
B	T	9,293	115	9,408	1·22
C	T	3,997	20	4,017	0·50
Total	T	14,912	195	15,107	1·28
D	C	1,070	1,976	3,046	64·87

TABLE 4

Showing amount of necrosis in treated and control plants in ORD. II

Plot	Treated or Control (T or C)	Number of healthy fruits	Number of diseased fruits	Total number of fruits	Percentage of necrosis
A	T	267	—	267	0·00
B	T	113	—	113	0·00
C	T	445	21	466	4·50
D	T	1,357	8	1,365	0·59
Total	T	2,182	29	2,211	1·31

The overall results obtained from the experiments on these two orchards (Table 5) show clearly that boron has a definite preventive effect on mango necrosis. Thus, among the treated plants out of a total of 17,318 fruits, 224 were necrotic, the percentage of necrosis being 1·29; among the controls out of a total of 3,408, 2,134 fruits were necrotic, the percentage of disease being 62·62.

TABLE 5

Summarized results of the incidence of necrosis in ORD. I and ORD. II

Orchard	Treated or Control (T or C)	Number of healthy fruits	Number of diseased fruits	Total number of fruits	Percentage of necrosis
ORD. I	T	14,912	195	15,107	1·28
	C	1,070	1,976	3,046	64·87
ORD. II	T	2,182	29	2,211	1·31
	C	204	158	362	43·65
ORDS. I and II	T	17,094	224	17,318	1·29
ORDS. I and II	C	1,274	2,134	3,408	62·62

Since there are a certain percentage of healthy fruits among the control trees the correction is applied statistically by the following equation :

$$p^* = c\,(1-c)p,$$

where p^* is the observed proportion healthy in the treated group.
   c is the proportion healthy in the control group,
   p is the corrected proportion healthy in the treated group.

Applying the above correction results are obtained as given in Table 6.

TABLE 6

Showing proportion diseased in ORD. I and ORD. II after statistical correction

Orchard	Proportion healthy in treated trees	Proportion healthy in control trees	Corrected proportion healthy in treated trees	Corrected percentage of necrosis in treated trees $(1-p)$ 100
ORD. I	0·9872	0·3513	0·9803	1·97
ORD. II	0·9869	0·5635	0·9699	3·01
Total	0·9871	0·3738	0·9794	2·06

The above table clearly shows that the percentage of necrosis in the treated trees of ORD. I is 1·97 when the controls are 100 per cent necrotic, i.e.

98·03 per cent fruits are prevented from getting necrotic by treatment with borax. Similarly in ORD. II 3·01 per cent of the treated fruits are necrotic compared to 100 per cent necrosis in controls and considering both the orchards together only 2·06 per cent of the treated fruits are necrotic.

In order to find out whether incidence of necrosis varies significantly in spite of the boron treatment the treated trees of plots A, B and C in ORD. I are considered alone (Table 7). The statistics are applied by the following equation :

$$\chi^2 = \frac{1}{p} \, N_i \, p_i - N = 87\cdot43,$$

where p is the total proportion diseased,

$\quad p_i$ is the proportion diseased in each plot,

$\quad N$ is the total number of diseased fruits,

$\quad N_i$ is the number of diseased fruits in ith plot.

TABLE 7

Incidence of necrosis in treated trees of ORD. I

Plot	Number of diseased fruits (N_i)	Total number of fruits	Proportion diseased (p_i)
A	60	1,682	0·035672
B	115	9,293	0·012224
C	20	4,017	0·004979
Total N =	195	15,107	$p =$ 0·012908

Probability of getting such a high value of χ^2 on two degrees of freedom is less than one in a thousand cases. So it is concluded that incidence of necrosis varies significantly from plot to plot and the efficacy of borax remains unchanged. Now it has already been pointed out that plots A and B are more affected than plots C and D in this orchard and that plot A is nearest to the operating brick-kiln on west. These results may therefore imply that the amount of boron required to prevent necrosis may vary with the intensity of the disease. No such statistical analysis was applied to ORD. II as all the plots were known to be uniformly affected and any difference in the incidence of necrosis after treatment may be attributed to undetermined factors.

DISCUSSION

Two heavily affected orchards situated at a distance several miles from each other were utilized to find out the effectiveness of boron in preventing necrosis. Although the general method was the same, unlike the previous

experiments (Das-Gupta and Sen 1960) where two concentrations of boron were applied, this year only 6 lb. of borax per 100 gallons were used throughout the experiments. The relative effectiveness of different concentrations of boron were not studied during the present experiments. The previous experiments had shown that there is no significant difference in the efficacy of borax when sprayed twice and once respectively. As adequate level of boron was the aim in the present experiments, trees were sprayed thrice (before flowering, during flowering and immediately after fruiting) with a view to gain more practical results, leaving the question of efficacy of more than one spray for future experiments. The location of the nearest brick-kiln to ORD. I and the wind direction was such that plots A and B were almost uniformly affected. A statistical analysis of the effectiveness of a given quantity of borax with varying degrees of air-pollution in different plots of ORD. I showed that efficacy of borax remains stationary as pollution increases, thus increasing necrosis. This result is in conformity with those obtained in the previous year (Das-Gupta and Sen 1960). The most important point however in the present study is the successful control of mango necrosis by means of borax spray. In a very large majority of fruits the disease was altogether prevented. This provides ample corroboration to the results obtained in the previous experiments (Das-Gupta and Sen, *loc. cit.*).

The prevention of mango necrosis by borax spray suggests a boron deficiency nature of this disease. Boron has become recognized as an essential plant food element during the recent years. While it is minor in the sense that small quantities are necessary, it is major in its essential role in normal plant nutrition. Boron deficiency has been found in all types of soils from light sandy soils to acid mucks. The element boron occurs naturally in all soils, but due to some chemical, bacterial or physical action it may not be available to plants. Naftel (1937) found that in high lime soil, boron was not as available as in low lime soil. The evidence obtained by him indicated the possibility of stimulation of bacterial action by liming the soil, so that there may be competition between plant and bacteria for nutrients. Results of various workers show that the fixation of boron in soil is chemical rather than biological (Midgeley and Dunklee 1939; Cook and Millar 1939; Tiulin 1940; Muhr 1940; Parks and Shaw 1941; Drake *et al.* 1941; Shive 1941; Reeve and Shive 1944). Fixation of boron may also occur due to various other factors like low moisture content (Brown and King 1940; Hobbs and Bertramson 1949; Woodbridge *et al.* 1952), length of day (Warington 1933), drought (Latimer 1941), climatic conditions (Eaton 1944) and many others resulting in its unavailability to the growing regions of plants. The authors suggest a disturbance in boron metabolism due to the interaction of certain brick-kiln fume constituents with the metabolic products of mango fruits and leaves to be the immediate cause of the disease and that boron applied in the form of

spray in adequate doses and appropriate time is a good practicable method of counteracting the deficiency and controlling mango necrosis.

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PREHORMONES

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Abstract

In the present state of our knowledge the status of many of the so-called 'hormones' is questionable; the peculiar 'latent period' in their action makes it possible that they are in reality 'prehormones' or 'precursors' of biologically active hormone molecules. The evidence bearing on this possibility is discussed in relation to the current interpretation of the term 'hormone'.

Introduction

The possibility of the existence in the endocrine glands of substances which are not quite the same as the conventional 'hormones', obtained by chemical processing of the glands, appears by and large to have been realized. Recently, Young (1959) has discussed the implication of this particular aspect of hormone research. After emphasizing the continued uncertainty about the identity of many known hormones he writes : 'Is it the substance that is present in the endocrine organ, or in the blood flowing from it, or is it the substance which may be formed by metabolic changes in the blood stream or in non-target organs from the material originally liberated by the endocrine gland ? Or is it whatever may ultimately appear in the tissue on which the hormones act and which may itself be capable of evoking a biological response in the tissue which the chemical substance in the endocrine organ or in the blood would not be capable of eliciting from an isolated tissue?'

The interrelationship of the structure and form of chemical moieties which the endocrine glands elaborate, store and release is not known. Further, it is possible that the material released by the endocrine glands in blood for transport to various parts of the body may be structurally different from that having specific hormonal effects on the target tissue. These are some of the basic questions which remain to be answered in precise terms by the future investigators. In this connection it is pertinent to mention that the factors now known as pepsinogens and trypsinogens are actually precursors of enzymes (preenzymes). The existence of these precursors was established at a much later stage in the history of enzyme research (*see* Northrop 1935 ; Neurath 1957).

The object of the present article is to stimulate thought on one such aspect of hormone research which has so far received only a scant attention.

I. 'PRE'-PROTEIN HORMONES

Precorticotrophin.—Dasgupta and Young (1958) demonstrated that a crude extract of fresh ox anterior pituitary tissue prepared in 0·9 per cent sodium chloride solution did not cause any depletion of adrenal ascorbic acid as tested by the method of Sayers *et al.* (1948). But such extracts acquired activity detectable by this test after the following treatments : (i) adjustment of pH to 3 by addition of 2N HCl, (ii) addition of urea to make 6·6 M solution and (iii) addition of sodium chloride to make a 5 per cent solution. An alkaline extract of ox anterior pituitary (Young 1944) devoid of adrenal ascorbic acid depleting activity behaved in an identical manner after pH adjustment and addition of urea. On the basis of these findings, these authors postulated the presence in the pituitary of a factor acting as the precursor of corticotrophin. They named this pituitary factor as 'precorticotrophin'. Dixon *et al.* (1959) recently confirmed the findings of Dasgupta and Young (1958) and attempted to activate precorticotrophin to corticotrophin by hypothalamic tissue and total adrenal extracts, but obtained negative results. Dasgupta (1960) observed that the precorticotrophin content of pituitary was nil when the gland was subjected to either drying with acetone (after mincing) or repeated freezing and thawing prior to homogenization. His explanation for this fact was that either precorticotrophin was destroyed completely or it underwent activation to corticotrophin by these treatments. He also discussed the possibility of release of this 'prehormone' into blood by the pituitary. Recently, he has been able to demonstrate the existence of 'precorticotrophin' in the normal rabbit blood serum (unpublished data).

On the basis of these studies it seems that 'precorticotrophin' acts as a physiological precursor of corticotrophin. However, it remains to be determined whether or not any of the *in vitro* activating processes studied by Dasgupta (1960) has any relation to the condition encountered *in vivo*.

Earlier, certain authors reported the presence in the blood of Addisonian patients of a principle which they termed 'activable corticotrophin'. They observed that this principle became active in the Sayers test only when treated with oxycellulose. They did not, however, consider the possibility of the presence in blood of a hormone precursor. However, the identity of the 'activable corticotrophin' with 'precorticotrophin' seems now to be a possibility. The experimental evidence on this point might even explain the cause of unusual dark pigmentation noticed in Addison's disease; a corticotrophin rich in MSH activity but relatively poor in corticotrophic activity may be released from the circulating 'precorticotrophin' under conditions of this disease.

In view of the findings of Dixon *et al.* (1951) that the alkaline extract of ox anterior pituitary contained a factor which caused a small but measurable

ponderal stimulation of adrenals of rats hypophysectomized 2-3 weeks previously, Dasgupta (1960) studied the effect of precorticotrophic extract under similar conditions. He observed that precorticotrophin did not have a direct influence on adrenal weight stimulation caused by the alkaline pituitary extract in the hypophysectomized rats. He pointed out that as a precursor of corticotrophin, precorticotrophin might have some role of an indirect nature.

Growth hormone.—Apart from stimulating growth, this hormone is known to produce a number of clearly defined physiological effects in experimental animals *but only after some latent period.* Young (1945) and others (Cotes *et al.* 1949; Park *et al.* 1952) showed that prolonged treatment with growth hormone in intact rat, cat and dog results in a diminished utilization of glucose and ultimately, under certain conditions, to frank diabetes, due to the formation of some inhibitory factor in the blood. Further, a growth-promoting action of growth hormone in isolated systems has not yet been demonstrated. The recent endocrine literature is deluged with papers describing one or other type of effect of this hormone (*see* Astwood 1955; Ketterer *et al.* 1957; and Randle 1957).

Recently, Young (1959) has briefly discussed the action of growth hormone on carbohydrate metabolism with a view to emphasize the fact that the hormone undergoes some metabolic transformations before it exerts its effect on the tissues. In other words, growth hormone in reality acts as a 'prohormone'. It is to be noted that unlike the other pituitary factors there is no specific target of growth hormone. It seems reasonable, therefore, to expect that future work will adduce experimental evidence to establish the 'prehormonal' status of growth hormone.

Melanocyte stimulating hormone (MSH).—The possibility of the existence of a precursor of MSH has been considered by Waring and Ketterer (1953). The MSH activity of the ACTH preparations from whole pig pituitary was potentiated by the addition of alkali, whereas crude or purified pig posterior pituitary extracts did not show any such potentiation.

Landgrebe *et al.* (1955) discussed the possible existence of a precursor of MSH in the anterior pituitary and seemed to favour the view that either a part or whole of the ACTH is the likely precursor of MSH (Waring and Ketterer 1953). Recently, Young (1959) speculated that in blood of Addisonians, where the corticotrophin (ACTH) level is high, substances like melanocyte stimulating hormone might originate as catabolic products of corticotrophin. In this connection it seems worth while to investigate the possible relationship of MSH with 'precorticotrophin' of the ox anterior pituitary.

Gonadotrophins.—It is currently believed that all the known gonadotrophins have a portion of their molecule in common (Lamond and Claringbold 1958). According to another group of workers (Aron and Aron 1957) there is only one pituitary gonadotrophin which produces FSH or LH effects according to

its blood concentration. Raacke *et al.* (1957) reported that the two observed effects of PMS gonadotrophin are, in fact, due to one molecule. On the basis of this evidence one can speculate about the existence of a mother molecule ('Pregonadotrophin') in the pituitary which may serve as the precursor of specific trophic factors (FSH or LH) depending upon physiological needs (*see* Selye 1947).

Cellular composition of the anterior pituitary.—The current view is that the three demonstrable cells of the anterior pituitary represent different stages in the cytomorphosis of a single type of cells. It has been shown that in rats there are two distinct types of chromophobes, one of which elaborates basophil granules and the other acidophil. At any rate, it seems far easy to conceive that the pituitary elaborates, stores and releases not as many different chemical principles as has so far been claimed. It is more likely that one or two highly labile molecules are discharged by the pituitary which undergo metabolic degradation during circulation and release hormonal material ready to be 'utilized' by the cells of the responsive target tissues. Alternatively, these native molecules may undergo some other change at the instance and site of the 'target' so that it (the target) can make use of the degradation products for its own synthetic purposes.

The idea that the pituitary is a kind of departmental store catering to the needs of the whole body in a ready-made form does appear to have a rather limited appeal in the present stage of our knowledge.

Oxytocin and vasopressin.—Abel and Nagayama (1920) were the first to conceive that one single large labile molecule having all the physiological properties (oxytocic, pressor and antidiuretic) was extractable from the posterior pituitary. Nearly two decades later, Van Dyke *et al.* (1942) obtained a protein preparation from the posterior pituitary and considered it to be of unitary nature. It possessed all the activities of the gland (cf. 'precorticotrophin' which is devoid of adrenal ascorbic acid depleting activity) in proportion to their occurrence in the gland. Van Dyke and Block (1950) determined the amino-acid composition of the posterior pituitary principle and calculated its molecular weight (30,000) which was in agreement with the value previously found by Van Dyke *et al.* (1942). A hypothetical molecule (molecular weight 30,000) consisting of one molecule of oxytocin and one of vasopressin would show biological activity comparable to that of the actual protein molecule separated by them.

In view of the fact that a native protein molecule is usually extremely labile it is not surprising that the Van Dyke protein was found to be electrophoretically inhomogeneous (Nord and Bier 1953). It is possible that under the action of the electrical field or even of the salt constituting the buffer the native hormone molecule suffers structural change. On the other hand,

since the supposed unitary substance of the posterior pituitary itself possesses hormonal functions (unlike precorticotrophin) it seemed possible to determine the unitary nature of the substance by means of countercurrent distribution.

In point of fact, Acher et al. (1955, 1956) employed countercurrent distribution in the solvent 2-butanol : 0·5 trichloracetic acid and showed inhomogeneity of Van Dyke protein. They also came to the same conclusion by the use of dialysis, electrodialysis and trichloracetic acid precipitation. In spite of what has been contended by Behrens and Bromer (1958) the present authors are inclined to favour the earlier idea of the singularity of Van Dyke protein because trichloracetic acid solution was one of the solvents employed. It may be mentioned in passing that Van Dyke et al. (1957) opined that the ox posterior lobe pituitary stores a protein containing the polypeptide.

Again, if one hormone is secreted into blood one stimulus is expected to evoke the gamut of physiological responses. That an overlapping of responses does actually occur has been shown by Harris and Pickles (1953) and Eranko et al. (1953). Coitus resulted in milk ejection as well as in antidiuresis in human being and the rat. However, Cross (1950) showed that the two different types of effects produced by a single stimulus, such as sucking the young, do not correspond to equivalent amount of specific principles liberated from the mother molecule. Landgrebe et al. (1955) argued that the target organ might deal with the hormone (oxytocin or vasopressin) differently from the protein molecule separated by Van Dyke.

However, it seems possible that the posterior pituitary may elaborate essentially one substance having all the known activities of the gland. Until a similar substance is shown to be present in blood, the question of its release in the same molecular form by the gland should be regarded as undecided.

Thyroid hormones.*—Perhaps the most illuminating example bearing upon the present theme of 'prehormones' is provided by the thyroid hormones. It is necessary to mention at the outset that the identity of the thyroid hormone remains as yet undetermined. For more than thirty years l-thyroxine was believed to be the only hormone to be secreted by the thyroid gland. Later, two independent groups of workers (Pitt-Rivers in England and Roche in Paris) established the presence in thyroglobulin of l-3, 4, 3'-triiodothyronine which is biologically several times more active than l-thyroxine. Triiodothyronine has greater diffusion rate than l-thyroxine and is present in blood in minute amounts. Subsequently Roche et al. (1954) reported the presence of some iodinated compounds of unknown nature in thyroid, bile and urine. In 1955 the Roche group separated two more new substances from the rat

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* Thyroid hormones are included under 'Protein hormones' for the sake of convenience of discussion.

thyroid gland, namely l-3, 3', 5'-triiodothyronine and l-3, 3'-diiodothyro-
nine, the latter having almost as much activity in goitre prevention assay as
l-thyroxine (*see* Roche and Michael 1955; and Pitt-Rivers and Tata 1959
for references). But compared with the action of adrenalin, even the most
potent substance triiodothyronine requires unusually long latent period (3
days) before it can exert its primary effect and that is, an increase in oxygen
consumption by rat kidney slices (Thibault 1957). Evidence like this ob-
viously points to the possibility that we are in fact dealing with precursors
and not the real thyroid hormones.

Recently, a formidable array of thyroxine analogues have been synthe-
sized and tested biologically in different laboratories. Some of the synthetic
compounds were even found to be active in evoking thyroxine-like responses
(*see* Salenkow and Aspar 1955 for references). But no compound should be
regarded as thyromimetic until it is demonstrated to produce all the physio-
logical activities of the thyroid. Nevertheless, the net outcome of all the
researches in this field has been that the substance stimulating the metabolic
rate of tissues is neither thyroxine nor triiodothyronine but perhaps some of
their degradation products. On the other hand, the new synthetic triiodo-
thyronine analogues like triiodothyroacetic acid ('TRIAC'), tetraiodothyroace-
tic acid (TETRAC), triiodothyropropionic acid (TRIPROP) and tetraiodo-
thyropropionic acid (TETRAPROP), which are believed to be produced in the
peripheral tissues, are currently considered as the probable active form of the
hormonal substances responsible for stimulating metabolic rate in tissues
(Thibault 1957). Therefore, so far as the thyroidal hormones are concerned
the identity of the real hormone(s) has still eluded detection; and the sub-
stances that we are dealing with at present may well be looked upon as 'pre-
thyroid hormones'.

Chromactivator hormone of the crustaceans, prolactin and insulin.—Evidence
for the existence of prehormones has been reported by Carlisle (1958) in crus-
tacea. He found that an inactive chromactivator was present in this group
of animals which could be activated into chromactivating substances by
boiling, addition of acetone, alcohol, trichloracetic acid, formalin or urea.
Carlisle (1957) opined that the lactogenically active form of prolactin is an
artifact prepared from the inactive material of the primary extract. Young
(1959) claims to have obtained evidence in support of the view of Himsworth
(1934) that insulin may be released by the pancreas into the blood in a form
not having the specific hormonal function. In other words, it may be that
the insulin secreted by the pancreas is in reality 'preinsulin' and that this
substance becomes hormonally active after it reaches the cell and impinges
upon extracellular-intracellular barrier. Krahl (1957) has tacitly expressed
a similar view.

II. 'PRE'-STEROID HORMONES

In the field of steroid hormones perhaps the most interesting instance of 'prehormones' is certain adrenocortical steroids which act as 'preandrogens',* since they do not possess androgenic activity as such but during metabolism are converted in part to androgenic substances. Cortisone and cortisol may be converted partly to androgenic steroids, 11-ketoandrosterone, 11 β-hydroxy-androsterone, 11 β-hydroxy-Δ^4-androstene-3, 17-dione, and adrenosterone (Dorfman 1955; *also see* Kar 1947). Similarly, 17-hydroxyprogesterone and 11-deoxycortisol yield the androgens, Δ^4-androstene-3, 17-dione and androsterone (Dorfman 1955). However, unlike other known 'prehormones' (e.g. 'precorticotrophin'), the biochemical mechanisms responsible for the formation of androgens from 'preandrogenic' adrenal steroids have been extensively investigated. Thus, Δ^4-3-adrenal ketosteroids of the C_{21} series may be converted to C_{19} steroids either prior to or after reduction of the Δ^4-double bond. Removal of a side chain before reduction of the double bond results in the formation of 11 β-hydroxy-Δ^4-androstene-3, 17-dione and adrenosterone and the subsequent formation of androgens, 11 β-hydroxy androsterone and 11-ketoandrosterone. The latter two androgens may also arise from side chain removal subsequent to ring A reduction (Dorfman 1955). A similar mechanism is responsible for the formation of androgens from 17-hydroxy-progesterone and 11-deoxycortisol. These steroids yield the androgens, androsterone and Δ^4-androstene-3, 17-dione. Even the enzyme systems with sterioisomeric specificity responsible for the changes in these 'preandrogen' molecules, leading to their transformation into androgenic substances, have been isolated (Dorfman 1955). It is interesting that a microbe *Fusarium solani* can convert a number of 'preandrogenic' C_{21} steroids into androgenic C_{19} substances (Vischer and Wettstein 1953). Admittedly, the scope of the term 'prehormones' has to be broadened a little in order to accommodate the 'preandrogens' because their mother substances like cortisone and cortisol have clearly defined physiological properties of their own. It may be recalled that the other known prehormone 'precorticotrophin' is physiologically inert *per se* (Dasgupta and Young 1958). Nevertheless, this difference does not limit the 'prehormonal' status of 'preandrogens' nor minimize the relevancy of their inclusion in the present discussions.

CONCLUDING REMARKS

From the evidence marshalled in this article it seems that the existence of the class of substances which could be fittingly designated as 'prehormones' is a strong possibility. In its broad sense this term refers to substances which

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* The term 'preandrogen' is considered to be more appropriate than 'proandrogen' (Dorfman 1955) at least in the context of the present discussions.

may give rise to 'hormones' with well-defined physiological properties. The present lack of knowledge about the physiologically active form of the so-called 'hormones' is perhaps the greatest of all limitations in our understanding of the mechanism of their action at the cellular level. Moreover, the current definition of hormone presupposes that *they should act instantaneously* and should not require any 'latent period' for eliciting the specific responses in their target tissues. But in reality many of the known hormones do not conform to this criterion because there is always a latent period before their action becomes manifest. This conundrum could be solved perhaps by an amendment of our existing knowledge about the *identity* of these hormones. It is possible that they are, in fact, precursors or 'prehormones' of the substances having the specific (and prompt) physiological activities.

The questions raised in the article are not easy to answer; but research on this aspect of endocrinology is expected to be rewarding and to yield results of far-reaching significance.

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ON THE METHODS OF STUDYING FLORAL MORPHOLOGY *

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ABSTRACT

Certain methods of approach, like the comparative, the anatomical, the developmental, the teratological and the historical ones, in regard to the study of floral morphology, including some factors relating to the comparative approach, are discussed and the implications of the so-called synthetic approach are analysed.

Intimately connected with the problem of flower morphology are the methods of studying it, for much of the controversies on the subject can be traced between the exponents of different methods of study. The most obvious of them are: (1) Comparative approach, (2) Anatomical approach, (3) Ontogenetic approach, (4) Palaeobotanical approach and (5) Teratological approach. It will be worth while to examine them briefly and to analyse their interaction upon one another.

Comparative Approach.—The method of studying external form, descriptive morphology, is the oldest and perhaps the most popular. For a considerable period of time this was the only method available for the study of plants and their organs; and it is amazing indeed how much knowledge and insight was amassed through this apparently simple method. Some authors have been so much influenced and charmed by this contribution that they have so restricted the scope of morphology as to be covered by the study of external form alone.† Very soon the external morphologist realized the importance and value of comparison in his studies. He began to understand and interpret objects he did not know in terms of those he knew. Thus were laid the foundations of *comparative morphology*, which even today is largely based on the study of external form but derived confirmation from all other branches of plant study. For this reason comparative morphology is sometimes considered as synonymous with external morphology. It is in this restricted sense that the expression is being used in the following description.

Notwithstanding the obvious shortcomings and limitations—external appearances are often deceptive!—of this method of study there are certain advantages that flow from it and that cannot be ignored. It is the simplest

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* Research contribution No. 26 from the School of Plant Morphology, Meerut College, Meerut.

† During recent years Professor W. Troll has been a great exponent of this method of study. In his voluminous writings he has devoted considerable attention to this.

of all methods. Besides training the eye to see things critically and scientifically it also puts us on our guard not to miss the wood for the tree—a danger that is so inherent in other more specialized methods of study. Coming to more specific points, the so-called classical concept of the flower and its component parts, the various systems of classification that have brought order out of chaos, are all based primarily on the study of external form. However, there are certain limitations to which pointed attention must be drawn here, as inadequate appreciation of these seems to have led to some confusion in the past.

Type Concept.—An essential feature of comparative morphology is that we have to have an object with which we are more or less thoroughly familiar and with which we can compare the object of our study. It is obvious that this known object cannot be the same for all the different categories of objects with which we have to deal; it has got to be different, for instance, in the cases of root, shoot, leaf, flower, floral parts, embryo sac, embryo, etc. In order to be of universal use as a standard, what can this known object be ? Obviously it cannot be a part of a living plant; nor can it be that of an extinct one. It can best be just a concept, a mental picture without having a physical basis whatsoever. This is what Goethe gave us when he expounded his concept of *Urpflanze* (literally, primitive plant), a type for all angiosperms. He also postulated type concepts for different major groups of plants, for buds and other lateral appendages (e.g. foliage leaves and floral parts), and so on.

This type concept is just a mental picture of the form underlying a particular group of plants or any particular structure in them. It is just an abstraction and does not have any historical basis and is thus free of time. It can profitably be used in comparative morphology and at the same time can help us keep away from involvement in phylogenetic speculations. But this requires some deliberate effort as there is certain inherent difficulty in the employment of this concept. For instance, ' when organisms (or organs), viewed under the type concept, can be arranged in a series according to their degree of approximation to the type, this succession has often been treated as being actually, as a matter of history, derivative' (Arber 1950, p. 159). Thus the concept is readily transferred from ' the plane of abstractions, where it belongs, to that of sensuous thinking—the plane of the visible and tangible' (Arber 1950, p. 61). This is what actually happened under the powerful influence of Charles Darwin (1859). The concept was so to say bodily lifted from its context and transformed into an historical ' ancestral form' with which it had nothing to do. And since this required much less of abstract thinking than the original concept, it was readily accepted by less discriminating of morphologists, thus causing a good bit of confusion and controversy.

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During recent years Troll is a great exponent of type concept but his approach is more elaborate and difficult and barring brief references by Arber (1937, 1950) and Wilson and Just (1939) no detailed account of his views is available in English. He considers that the flower can be treated as (1) a biological type, (2) an organization type or (3) a *Gestalt* * type. It is with the *Gestalt* type that he concerns himself. His main emphasis, according to Arber (1950), is on form as seen by the naked eye and not on organization which is the main concern of the orthodox morphology. Troll represents the form of the reproductive phase of angiosperms with ' flower Gestalt'. This may be in the form of a simple flower (euanthium), an inflorescence (pseudoanthium) or an inflorescence of inflorescences. Thus the flower in Gestalt sense does not have a uniform organization, but may be constructed in diverse ways out of simple phyllomes or shoots.

Arber (1937, 1950), who has analysed the type concept in great detail, has drawn attention to practical difficulties inherent in Goethe's and Troll's concepts. She has, therefore, suggested an easier and more realistic notion of *parallelism*, which according to her may absorb both the type and Gestalt concepts. She believes (1950, p. 159) that ' The change from the type concept to that of parallelism has the advantage of detaching morphological thought from the tyrannic notion of irreversible historic derivation '.

In these short pages we cannot obviously enter into details of the subject, but it must be pointed out that the concept of parallelism too is not free from the implications of polyphylesis which if taken to extreme may not lead us anywhere.

Terminology.—Another important heritage that we have received through comparative morphology is a rich vocabulary of terms which has been used extensively by workers in different disciplines of botany. It must be remembered that almost all our descriptive terms were coined at a time when the entire knowledge about plants was primarily based on external morphology. They were so to say coined to serve the purpose of descriptive botany in which the plant was considered as consisting of so many different parts and not as a single whole or an organism. Needless to say that this purpose was admirably fulfilled by the terms coined.

Today the plant is studied from diverse points of view by experts who style themselves as morphologists, anatomists, morphogeneticists, palaeobotanists, cytologists, etc. All these have been using the terminology of

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* It is difficult to define the term *Gestalt* in English, but Arber's analogy (1950, p. 144) may be helpful. ' Suppose we imagine a group of art students, with a model posed before them, and suppose that one of them produces his impression of the figure in a charcoal outline, one as a water-colour, one in carved wood, one in a clay model and so on. The results—since they are not produced in the same medium, and are thus built up out of different elements—cannot be said to belong to the same organization type; but since they are all manifestations of the same model, they fall into the same *Gestalt* type.'

the descriptive botany, which no doubt is very inadequate and ineffective for their specialized objects. We do not very much mind this fact for the simple reason that we have learnt to grow with this terminology and we frequently understand by it what we mean in a general way. But every one of us is familiar with the annoyance that some of these terms cause us at times. An example will be helpful. Let us consider a simple term, such as ' leaf '. Everybody uses it and is more or less familiar with its implications. However, every anatomist or a developmental morphologist knows only too well how difficult it is to demarcate limits between a leaf and the stem on which it is borne. In fact he sees full structural continuity between leaf and its stem; for him there exist no limits and actually there are none. So if he were given an option to coin a term for this expanded structure he would have perhaps liked to call it by some other name, implying in some form or another the important result of his study. The same could have been the case with flower, floral parts and other structures. So the entire terminology from this point of view would have been different. But this is hardly desirable. We know too well that terminology is not the end-all of our science; it is just a means of communicating our ideas and observations to others. With this background the terminology of the external morphology has been retained by different branches in botany. But this must be used with clear knowledge of limitations of the various terms and any controversy around such terms or categories should be considered as futile.

There are, however, certain terms and expressions that have been used in floral morphology in somewhat confusing manner. *Cohesion, adnation, suppression, chorosis, splitting, lobing*, etc., for instance, are frequently used in two senses, ontogenetic and phylogenetic. It is in the latter sense that they have not been correctly understood. When we say, for instance, that the petals in a particular plant show *phylogenetic* cohesion, we do not actually mean that they have undergone this change from freedom to fusion *at any time of their existence, past or present*, during the ontogeny of some one individual. It is simply an expression that enables us to explain the condition in one species in terms of another where the parts are free; these are concepts of idealistic approach (according to some of phylogeny) rather than facts of ontogeny and constitute a useful language of comparative morphology.

The nineteenth century saw great diversification of morphological study. With the improvement in technique and instrumentation newer avenues were opened for studying plant morphology. These led to what may for the sake of description be described as *anatomical* approach, *developmental* approach, *teratological* approach, *historical* or *palaeobotanical* approach, etc. There is little doubt that these diverse approaches can also be comparative in their outlook and can be treated under comparative morphology in a wider sense. But as stated earlier we treat them separately.

Anatomical Approach.—Notwithstanding the fact that many earlier investigators (e.g. Brown 1833; Darwin 1862) made use of vascular bundles in the interpretation of flowers, van Tieghem (1868, 1875, etc.) is generally regarded as the founder of the study of floral anatomy. It was indeed he who recognized this as a fruitful branch of study and through his extensive researches raised it to a place of distinction. The school of van Tieghem in France, followed by that of Eames in America and the extensive contributions of Miss E. R. Saunders and Mrs. Agnes Arber at Cambridge, have given this anatomical method the recognition that appears to be its due. As a result of active work at these centres and elsewhere, a considerable amount of literature has accumulated and claims have been advanced, time and again, that the arrangement, number disposition and course of vascular bundles provide significant and reliable clues to the puzzling variations in the external form of the floral structures.

The usefulness of the anatomical method lies in the fact that the vasculature of the flower—the number, arrangement and disposition of vascular bundles in different floral parts—is a fairly constant feature and sometimes it may be more or less distinctive of certain groups of plants or even species. Besides, as the vasculature of the flower is not subject to the same spatial difficulties as is the external form of its organs, it may give us better information about the morphology of the organ in question. For instance, where stamens are epipetalous their bundles may be quite free from those of the petals, indicating their separate identity. Then in some cases vasculature of the flower may be more or less modified through such processes as adnation, cohesion, reduction, amplification, etc., and may provide valuable material for comparison. Anatomical method, since it is concerned with the study of mature organs, has often been used for checking and verifying the inferences based on comparative morphology.

Other claims regarding the conservative nature of the vascular tissue and the rôle of vascular bundles in the solution of the problem concerning the nature of the wall of inferior ovary are, in our opinion, not well founded (*see* Puri 1951).

Some critics of the anatomical approach have chosen to regard it as an entirely useless pursuit. It is often asserted, for instance, that 'strands are formed where major nutritive movements are promoted as growth proceeds and that the presence or absence of strands in various groups and situations throughout the floral organ is purely consequential to the situations and activities of the organs themselves' (Thompson 1936). Those who have concerned themselves with the method know that this is not a fair criticism. It is likely that this was a reaction to the exaggerated claims of some floral anatomists who thought rather too much of the method of their choice (*see* Puri 1951; Hall 1956).

Developmental Approach.—It was the powerful personality of Schleiden (1849) that changed the course of botanical studies about the middle of the last century by advocating rather strongly the cause of developmental approach. But actually it were the patient efforts of men like Payer (1857), and more particularly Hofmeister that laid the foundations of developmental morphology. In the present century Goebel (1933) had been one of the strongest exponents of this method of study.

In this method the main emphasis is on the ontogenetic stages* that lead to the mature condition. No doubt it offers valuable data for comparison of developmental pattern of the *same* organ in different existing species or sometimes of different organs. For example, in maize the early stages in the development of tassel are surprisingly similar to those of ear although the end products look so different at maturity (Bonnett 1948 and others). In the same way similarities in the modes of development of foliage leaves on the one hand and floral organs on the other have been emphasized by a number of workers (*see* Tepfer 1953; Tucker 1959). Such comparisons, however, may not always be quite dependable for basing morphological interpretations, for morphologically different organs may sometimes show similar developmental stages in their ontogeny.

Some developmental morphologists have also studied carefully the layer or layers at the growing apex actively involved in the initiation of different organs. This in itself is a useful pursuit. But when results of this nature are used for discussing questions involving morphology of the organs concerned, such an attempt leads to confusion (witness the excellent work of Satina and Blakeslee (1941, 1943, etc.), on *Datura*, where on similar findings they interpreted the sepals and petals as of foliar nature and stamens, carpels and ovules as of axial nature as they initiate from deeper layer).

Developmental morphologists have also been attempting to explain plant form in terms of *cause* and *effect*, the word cause being used only in proximal sense involving *physical* and *chemical* factors only. Through their laborious studies they have been able to present a more or less complete account of the developmental history of various organs or structures and have thus been able to explain *how* a particular situation may have arisen. The sponginess of the mesophyll tissue in a leaf, for instance, has been explained as being brought about by unequal growth and enlargement of the cells of the lower epidermis and those of the internal tissue. Apparently this is a satisfactory explanation of the mechanism of growth, but nobody would logically claim it to be the *cause* of sponginess. So causal morphology as we understand it today deals with the conditions of growth rather than

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* For the sake of convenience of description the ontogeny is distinguished into separate stages but actually speaking it is a continuous process.

its real cause. Perhaps our methods are too crude and our approach too superficial to determine the cause behind a particular form. That subject is just outside the scope of science. We are perhaps just comparable to an ant that crawling on a piece of printed paper sees some letters and ascribes them to the iron pen without seeing the hand behind the iron pen! But under the present state of our knowledge we have reason to be satisfied with what little progress we are making in this new field which does not really belong to pure science.

Recently developmental morphologists have been devoting considerable attention to what may be called the *experimental approach*. They have devised ingenious techniques and methods involving tissue culture and surgical operations for studying plant behaviour under controlled conditions. Although still in its infancy this branch of study has yielded useful information as to the conditions of growth and has thus given us better appreciation of the plasticity of the living matter. But the data that is fast accumulating is perhaps as difficult of a morphological interpretation as that obtained from studies of teratological specimens occurring in nature.

Teratological Approach.—Plant morphologists have ever been interested in studying teratological (Greek *teras* or *terat* – wonder) specimens. Even lay people who have little or no interest whatsoever in the 'normal' forms have always shown an abiding interest in abnormal structures. I recall with joy the great excitement with which my little daughter, when she was just five years of age, drew my attention to a 'double' petunia flower in a bed of 'single' flowers. Such excitements are familiar to most of us.

As the so-called normal form is itself so variable, it is difficult to define what exactly constitutes an abnormality, monstrosity or malformation. Some authors describe it as 'any extraordinary modification in the formation or the development of organs irrespective of any influence upon health', while others consider it as some 'considerable deviation of structure generally injurious or not useful to the species'. As a rule, therefore, it may be characterized as organ or part having undergone conspicuous change of structure and form and sometimes of function. It is not always quite possible to distinguish malformation from the appearance of disease.

It is equally difficult to classify these malformations since every specimen represents its own type and there are so numerous of them. But *prolification, phyllody, fasciation* and *hypertrophy* are some of the common phenomena that are responsible for abnormalities in form and structure of inflorescences, flowers and their parts.

Malformations are known to be caused both by *internal* and *external* factors. Those caused by internal factors are often spontaneous and heritable to a greater or lesser extent. The 'doubleness', for instance, in *Petunia, Antirrhinum*, etc., is heritable (?) and so is fasciation of the inflorescence in such

cases as *Celosia*, even though some individuals in the progeny may not show it. Among the environmental factors nutrition has often been considered as very important.

Many instances are known where malformations are *apparently* caused by infection of some foreign organism such as a fungus, a bacterium or an insect. Infection by *Cystopus candidus* may cause all sorts of modifications in floral parts in *Brassica* and other crucifers. In some cases mechanical injury is known to cause fasciation. In *Nasturtium*, for instance, 60 per cent of the germinating seedlings from which epicotyle was removed developed into fasciated individuals. In other cases, however, e.g. *Oenothera*, Primroses, etc., mechanical injury at any stage of development failed to produce the desired effect.

There have been considerable differences of opinion as to the significance and value of teratological studies. Some authors read in them reversion to ancestral characters and regard them as ' one of the happiest hunting-grounds in which to track down ancestral characters'. But some others discard them completely as being merely freaks of nature and not deserving any attention.

It is true that many of the abnormalities fit in well with the general pattern of evolution of an organ that we may frame for ourselves. But there are many other abnormalities that do not fit in this way. They are therefore considered as 'incidental' and not of much consequence for the simple reason that if taken into account they may take us too far and may prove what we do not want to prove. In view of such considerations we are inclined to agree with Arber (1931) in not attaching any morphological significance to abnormalities.

Notwithstanding all this, teratological specimens are in themselves interesting objects for study. They do reveal the plastic nature of the living matter and this in itself is a fascinating pursuit for study. They show us what an organ ' can do' if not what it ' has been'.

Historical Approach.—This method of approach is based on the assumption that during the last 100 million years or so—for which period the angiosperms have been in existence—the form and structure of angiosperm flower have undergone certain changes and modifications and that all the different forms that we see today have some connection—remote or close—between them. The succession of changes envisaged is further expected to be occurring in the form of fossils; only we have not been fortunate enough to discover them as yet. It will be seen that this approach is quite different from the typological one of comparative morphology. It has a physical or a materialistic basis and does not involve any mental picturing as does the latter. In order to distinguish it from the so-called classical or old morphology based on typology, Thomas (1932, 1950) described it as ' New Morphology'. Lam

(1948) is another strong exponent of this approach. But it must be pointed out that these two approaches are not miscible; attempts to mix them up in the past have resulted in much fallacious reasoning and bitter controversies.

This historical approach to the study of morphology should certainly appeal to everybody who has faith in the fact of evolution. In the domain of Pteridophytes and Gymnosperms it has contributed substantially towards a better understanding of these groups (witness the classical researches of Professor W. Zimmermann (1949, 1959) and Professor R. Florin (1951), among others). But we have very little useful data concerning the angiosperms and much less about the flower. Whatever may be the reason, we have almost no material of the so-called 'missing links' to study. So all that has so far been said concerning the morphology of angiosperm flower by the exponents of the 'new morphology' is largely based on circumstantial evidence derived from comparative study of living plants.

One of the important contributions of this branch of study is the telome hypothesis which was first formally enunciated in connection with lower vascular plants (Zimmermann 1930, 1959) but which has subsequently been applied to all groups. According to this view green algae with dichotomously forked and radially symmetrical thalli are believed to have given rise to primitive land plants with much branched axis and little differentiation. These in turn have produced the higher vascular plants with well differentiated root, leaf and stem. Thus the axis, the leaf, the sporophyll, the sporangia are all believed to have developed from the ultimate branches (*telomes*) through a number of evolutionary processes such as overtopping, planation, webbing, fusion, reduction, recurvation, etc. Evolution is further believed to have progressed along three main lines resulting in three main groups, Lycopsida, Sphenopsida and Pteropsida including ferns, gymnosperms and angiosperms (Zimmermann 1949).

It is true that some of the observed facts in lower vascular plants fit in very well with this hypothesis, but it meets serious difficulties in angiosperms. The only evidence that is cited in support of it from this large group is the occurrence of more or less frequent dichotomies of ultimate vascular veins in leaves, floral parts, etc. (*see* Lam 1948; Hammen 1948, etc.). These are considered as relics of ancient dichotomous branching. To many of those who are concerned with the so-called 'angiosperm-centered' morphology this interpretation of the open dichotomous endings of vascular veins appears to be least convincing. The situation, according to them, can be explained in a different and perhaps more effective manner. It has been suggested, for instance, that 'the dichotomous venation so commonly seen in cataphylls, bracts, and petals may largely represent, from an ontogenetic point of view, an 'arrested development' of vascular anastomoses . . . .' (Foster 1950).

Serious attempts have been made to interpret the stamens (Wilson 1937, 1942, 1950, 1953) and carpels (Hunt 1937, Wilson 1942) in terms of telomic hypothesis. But there is hardly any data that can be relied upon in this connection (*see* Puri 1947, 1951; Parkin 1951; Canright 1952).

Thus while historical approach is as valid a pursuit of studying plant form as the idealistic method, we are limited here by the paucity of adequate material. Despite the best attempts of palaeobotanists all over the world we do not have any information about the so-called 'missing links'. If they are at all present they seem to be eluding us all this time as it were. It was this fact which was referred to as an 'abominable mystry' by Charles Darwin about 100 years ago.

Synthetic Approach to Flower Morphology.—These different approaches are all useful tools in the hands of the floral morphologist. They lead him to his goal in much the same way as do the different religions or political ideologies practised by mankind. They represent different paths and focus attention on different facets of our objective. Consequently every one of them reveals only partial truth and never the whole. If some people think otherwise they are obviously mistaken and are doing as much disservice to the method of their choice as those who discard it completely.

During recent years there has been much talk of a synthetic approach to the problem of floral morphology. I wonder if the implications of this plea are clearly understood by those concerned with the problem. To some it seems to mean with some people that the inferences and conclusions arrived at through one type of approach should also be checked and verified through another type. For instance, it is argued by some developmental morphologists, that to be acceptable the classical interpretation of the flower and its parts, that is based on comparative morphology and supported by floral anatomy, should receive support from ontogenetic studies as well. And when they find such support not forthcoming they advocate the discard of the classical concept as a useless hypothesis (*cf.* Grégoire 1938). In our estimate this is like testing the gospels of one religion or one political philosophy on the basis of another. And it is common knowledge that such a treatment is bound to result in confusion and chaos. Consequently such an understanding of the synthetic approach is basically wrong and will not lead us anywhere.

We, on the other hand, believe that any morphological conclusion arrived at in a certain field of study (provided, of course, it is based on sound reasoning) is valid within the limits of that field as this is conditioned by certain basic assumptions inherent in that method of approach. It cannot be confirmed or rejected on the findings from another field of study. Activity in different branches of study has brought out some partial truths about the object of our interest. These are inclusive rather than exclusive of one another. By

synthetic approach we should, therefore, understand the piecing together of these partial truths obtained from different fields of activity with a view to have a glimpse of the greater truth or the whole reality. Whether we can achieve this is largely determined by our power of comprehension.

Let us refer briefly to some obvious examples to illustrate the point at issue. Professor Troll's concept of the carpel being a peltate structure is not the same as the classical concept. In enunciating this he goes only so far as his ontogenetic findings take him. A classical morphologist, however, goes further beyond and visualizes Professor Troll's peltate carpels in terms of his basic 'type'.* Troll's concept, therefore, is more realistic but less idealistic and consequently less comprehensive than the classical concept. But it is not antagonistic to it; rather it is inclusive with it and both concepts can coexist.

In the same way during recent years two apparently different interpretations of angiosperms leaf have been offered in the form of partial shoot hypothesis and telomic hypothesis. These too are not antagonistic to one another but represent different facets of the leaf and hence we do not have to accept one and discard the other. Every one of them has some partial truth and none the whole. While one of the interpretations is idealistic and based on comparative morphology the other is historical and based on fossil record for whatever it is worth. They both have right to exist, even though students of leaf ontogeny may not find support for either of them.

Thus it is not proper for workers in one field of study to check and verify the conclusions and inferences based on study in other fields. This is no pleading for a licence for unbridled speculation. Workers in their respective fields will exercise better control in this respect and I will in this connection particularly commend the suggestions of Hall (1956) to workers in every field of enquiry although he addresses them to his own kith and kin. Every branch of study has some truth to offer and none has the whole. Let this partial truth be sieved out and, whenever possible, synthesized together with other partial truths towards a more complete realization of our objective. Such an attitude will eliminate controversies and will be conducive to more harmonious relations between workers in different fields and thus lead to clearer thinking.

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* For a detailed consideration of 'type' *see* Arber (1950).

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INDUCED MUTATIONS, EVOLUTION AND WHEAT BREEDING

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ABSTRACT

Owing to its ability to tolerate whole- and part-chromosome deficiencies and owing to the availability of extensive data concerning the location of genes on specific chromosomes through monosomic analysis, bread wheat offers an interesting material for mutation research.

The important features of the response of bread wheat to mutagens are: (*a*) phenotypic changes occur in M_1; (*b*) chlorophyll mutations are rare in M_2; (*c*) viable mutation frequency is high in M_2 but the spectrum of the induced mutations is very narrow and (*d*) aneuploids occur frequently in the M_2 and subsequent generations and complicate the calculation of mutation rate.

Mutation breeding offers considerable scope for incorporating a desirable additional attribute in an improved strain of bread wheat. A new fully awned strain named N.P. 836 has been produced from the awnless variety N.P. 799 in this way. For success in the production of new varieties through the use of induced mutations, it is necessary to subject the population to conventional breeding methods. Recurrent selection has a positive effect in advancing the yield of an induced mutant.

Among the three genomes of bread wheat, the D genome contributed by *Aegilops squarrosa* is relatively more radiosensitive and this genome appears to have introduced into bread wheat a physiological state which promotes the incidence of a higher frequency of chromosomal aberrations and viable mutations following treatment with mutagens. Among the hexaploid wheats the frequency of mutations is high in free-threshing spring wheats and low in the spelt, winter wheats.

Among the hexaploid wheat species, all except *T. spelta* give rise to *aestivum* type of mutants. The *spelta* origin of *T. aestivum* hence seems unlikely and it is suggested that *aestivum* and *spelta* might have arisen independently through chromosome doubling in hybrids between tetraploid species with and without the '*Q*' factor and *A. squarrosa* respectively.

I. INTRODUCTION

Bread wheat (*Triticum aestivum* L; $2n = 42$) has several favourable features which render it an ideal material for mutation research. First, it is a hexaploid in which gametes with either whole- or part-chromosome deficiencies are viable; in fact nullisomics as well as genomic substitution are tolerated. Riley and Chapman (1958) and Sears and Okamoto (1958) have recently shown that the reason for bread wheat behaving cytologically as a diploid, in spite of the existence of extensive segmental homology among the chromosomes of the A, B and D genomes (derived respectively from *T. aegilopoides, Aegilops speltoides* or some other member of the *Sitopsis* Section of

Aegilops and *Aegilops squarrosa*), is the presence of a multivalent suppressor gene system in chromosome V (chromosome 5B according to the new system of nomenclature proposed by Sears 1959).

Secondly, thanks to the development of a complete set of monosomics in the variety Chinese Spring by Sears (1954) we have now extensive data concerning the location of genes on specific chromosomes and of the homologous relationships among chromosomes belonging to the different basic genomes.

Thirdly, both chromosome length and DNA content data reveal that the ratio of the total chromatin length in diploid, tetraploid and hexaploid wheats is not of the order 1 : 2 : 3 but is about 1 : 1·5 : 2·0 (Marshak and Bradley 1944; Swaminathan and Natarajan 1957; Bhaskaran and Swaminathan 1960). The total length of the chromosome complements of *T. monococcum*, *Aegilops speltoides* var. *ligustica* and *A. squarrosa* vary in the ratio 1 : 1·24 : 0·71 (Pai and Swaminathan 1960). Sears (1959) has assigned each chromosome of the bread wheat variety Chinese Spring to its respective genome and he (1954) has also calculated the length of each of the 21 chromosomes. The proportion of the total lengths of the chromosomes belonging to the A, B and D genomes, as calculated from his data, falls into the ratio 1 : 1·28 : 0·84. There is thus a close similarity between this ratio and that recorded by comparing the total chromosome length of *T. monococcum*, *A. speltoides* and *A. squarrosa*. However, since the total chromosome length and DNA content of *T. aestivum* are only twice as great as that of *T. monococcum* and not three times as would be expected from considerations of chromosome number and the additive value of the total chromosome length of the three genome donors, it seems likely that chromosome diminution has occurred in the tetraploid and hexaploid wheats during their evolution (Pai and Swaminathan 1960). In other words, both emmer and bread wheats appear to be capable of withstanding a considerable degree of chromatin elimination and karyotype repatterning.

Finally, wheat is one of the crops in which intensive breeding work has been in progress in India since the last four decades (Pal 1954). The relative usefulness of hybridization versus mutation breeding for the purpose of incorporating a specific attribute in a highly-bred strain can hence be evaluated critically in this crop.

In view of the above considerations and encouraged by Gustafsson's (1947) statement that in bread wheat ' with suitable X-ray doses, a massmutating effect sets in ', mutation research was initiated in *T. aestivum* at the Indian Agricultural Research Institute in 1955. Some of the theoretical principles and practical results which have emerged from this research are summarized in this paper.

II. Mutation Characteristics of Bread Wheat

(a) Phenotypic changes in the M_1 generation

In the wheat seeds subjected to treatment with mutagens, 3 to 4 ear primordia are already differentiated and each ear appears to develop from one initial cell. This feature together with the ability of bread wheat to tolerate part- and whole-chromosome deficiencies renders the occurrence of phenotypic variation possible even in the M_1 generation (the term M_1 is used in this paper to denote the first generation of the treated material irrespective of the mutagen used). MacKey (1954a) has observed that speltoid chimeras in M_1 have about the same dose relationship as the total frequency of observable mutations in M_2.

(b) Mutation frequency in the M_2 generation

(i) Chlorophyll mutations

Stadler (1929) examined the frequency of induced chlorophyll mutations in species of *Triticum* and *Avena* in relation to the level of ploidy and observed a sharp decrease in the rate of chlorophyll mutations with increasing chromosome number. This finding has later been confirmed by Müntzing (1942), Fröier (1946), Smith (1950), MacKey (1954a) and Natarajan *et al.* (1958). This is because in a polyploid a loss or change at one locus is effectively buffered by the presence of loci with similar function in the chromosomes of the other genomes. The extent of this effect will, however, vary in the different types of polyploids and also among different strains of a polyploid species depending upon the degree of diploidization involved. Thus, among the several varieties of bread wheat studied at the I.A.R.I.. the Punjab wheat C-591 gives rise to *albina* mutations frequently while such a mutation never occurs in the others. None of the 21 nullisomics in wheat shows lack of chlorophyll development thereby making it evident that two or more chromosomes will have to be affected before any mutation relating to chlorophyll development can find phenotypic expression. The relative ease with which *albina* mutations can be induced in the variety C-591, however, suggests that in this variety only a single gene may be concerned with this character.

(ii) Viable mutations

The correctness of Gustafsson's statement that bread wheat mutates readily on being exposed to radiations has been borne out in subsequent experiments (MacKey 1954a, 1959; Natarajan *et al.* 1958; Pal *et al.* 1958; D'Amato and Moschini 1959). From such data, MacKey (1954b) and Swaminathan (1957) have remarked that the scepticism of Stadler (1930) with regard to the suitability of polyploid plants in mutation research is no longer

tenable. The mutation rate recorded in the wheat variety C-591 following treatment with P^{32} (Fig. 1), S^{35}, X-rays, fast neutrons and groundnut oil is given in Table I. The groundnut oil treatment was prompted by the finding that this oil is able to induce chromosome aberrations in several plants (Swaminathan and Natarajan 1956 and 1959). The types of mutations induced by these treatments and their relative frequencies are indicated in Table II. The data reveal that (1) in general the same types of viable mutations occur in all the treatments; (2) though a large number of viable mutations are produced, the mutation spectrum is very restricted and (3) a vast majority of the induced mutations relate to either the loss or the duplication of the speltoid suppressor gene ' Q ' situated in the distal end of the long arm of chromosome 5A. Our observations agree with the finding of MacKey (1959) that except for the occurrence of awns and adventitious spikelets, all mutations in bread wheat can be classified as mere shortening, lengthening, thickening or thinning of already existing organs or as physiological changes. Aneuploid, particularly monosomics—occur frequently in the M_2 and subsequent generations and speltoids associated with aneuploidy show an erratic breeding behaviour. Awn mutations (Figs. 2 and 3) occur readily in awnless varieties with a single gene epistatic mechanism of awn suppression (Pal *et al.* 1957). From the phenotype, the genotype as regards the awn inhibitor genes can be readily judged (Watkins and Ellerton 1940). Thus, long tipped varieties have the B_1 gene on chromosome 5A, short tipped ones have B_2 on chromosome 6B and completely awnless strains have either B_1 and B_2 or B_1 and Hd (located on chromosome 4B) or B_1 and B_2^a (an allele of B_2 which makes the plant half-bearded, Sikka *et al.* 1959). In mutation experiments with wheat varieties having different genotypes as regards the awn character, it was found that fully awned plants can be easily isolated in varieties with a single epistatic gene (Table III). Where two genes are involved it may

TABLE I

Mutation rate in the variety C-591

Mutagen	Dosage	Number of M_2 plant progenies examined	Number of mutations observed in M_2	Mutation rate per plant progeny (%)
P^{32}	5 µc per seed	92	39	42·4
S^{35}	,,	100	94	94·0
X-rays	11,000 r	60	29	48·3
,,	16,000 r	43	23	53·5
,,	22,000 r	27	6	22·2
Fast neutrons	10^9/cm²/sec. for 3 hrs	107	23	21·5
Groundnut oil	Soaking for 24 hrs.	29	44	155·5
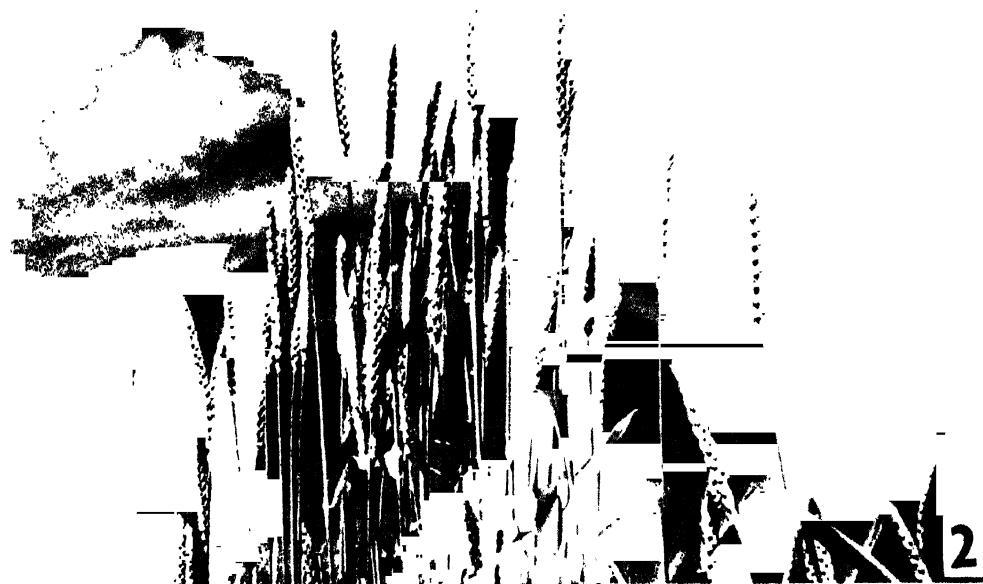
be possible to detect a fully awned plant only if a large population is raised; alternatively mutations affecting a single gene can be first isolated and such genotypes could again be subjected to irradiation.

TABLE II

Types and relative frequencies of mutations isolated in M_2 progenies of C-591

Mutation	Number of mutants and % of total mutations				
	P³²	S³⁵	Fast neutrons	X-rays 11,000 r	Groundnut oil
albina	0	0	9 (39·51)	0	0
Short and stiff straw	1 (2·51)	1 (1·06)	0	4 (13·8)	9 (20·45)
Fine straw	0	0	0	8 (27·60)	0
Speltoid	15 (38·65)	41 (43·62)	3 (13·17)	8 (27·60)	24 (54·54)
Compactoid	5 (12·82)	17 (18·09)	3 (13·17)	4 (13·8)	1 (2·27)
Lax ear	1 (2·51)	3 (3·18)	0	2 (6·90)	0
Awn character	9 (23·07)	21 (22·33)	6 (26·34)	1 (3·45)	2 (4·54)
Others*	8 (20·08)	11 (11·70)	2 (8·78)	2 (6·90)	8 (18·18)

* Include grass clumps, early and late types, colour and hairiness of glumes and grain colour.

TABLE III

Frequency of awn mutations

Variety	Awn character		Percentage of mutations at the locus		
	Phenotype	Genotype	B_1	B_2	Hd
N.P. 797	Long tipped	$B_1 B_1$	5·1	—	—
N.P. 798	,, ,,	,,	6·6	—	—
N.P. 799	,, ,,	,,	5·6	—	—
N.P. 809	,, ,,	,,	4·7	—	...
N.P. 790	Awnless	$B_1 B_1$ $B_2^a B_2^a$	0	0	—
H. 389	,,	$B_2 B_2$ Hd Hd	—	0	3·14

In fully awned varieties like C-591, awnless mutants occur rarely. These seem to arise from the deletion of the basic awn development genes since in genetic studies such awnless plants behave as recessives (Swaminathan and Natarajan 1959). On the other hand, when dominant epistatic genes are involved, the awnless condition is dominant in the F_1. Thus, induced awn mutations have helped to clarify the dominant relationships among the awn promoter and suppressor genes. It is also of interest that all the awnless wheat varieties subjected to monosomic analysis have been found to be awnless due to the presence of dominant epistatic genes and not due to the lack of awn development genes. Sears (personal communication) has expressed the view that deficiencies of awn promoters must have occurred in the history of wheat much more frequently than have awn inhibitors. The reason why none of them has been retained must be that such deficiencies result in too great a reduction in yield. This inference is supported by the observation of Swaminathan and Natarajan (1959) that in the variety C-591 awnless mutants are characterized by an appreciable reduction in the number of tillers and grain yield per plant.

III. Induced Mutations and Wheat Breeding

A comparative study of the relative efficiency of mutation breeding and of hybridization followed by backcrossing has shown that the former is more speedy and effective for incorporating the character of awning in tipped varieties. Several fully awned mutants have been isolated in the tipped varieties N.P. 797, N.P. 798, N.P. 799 and N.P. 809 and some of these awned mutants have been found to perform as well or even better in comparison with the controls in yield trials conducted during the last 3 years. An awned mutant of the variety N.P. 799 has been ranked first in yield trials conducted at the Botanical Sub-station, Pusa, during 1958 and 1959. This mutant resembles closely the parent strain in other morphological characters, maturity period, grain shape and quality and rust resistance. In genetic studies, it behaves as a single gene mutant. It has been named N.P. 836 and will be tested for its performance in several parts of Bihar and West Bengal during the *rabi* season of 1960. Its release for general cultivation in replacement of the parent strain seems a distinct possibility and will be welcomed by our farmers who prefer the awned wheats on the belief that they are less prone to bird damage.

MacKey (1954a) was the first to show by a mutation experiment that awns may have a positive function; the bearded mutant obtained by him in the variety Scandia-III consistently out-yielded the mother strain in trials carried out over a period of five years. A similar conclusion had been reached by Miller *et al.* (1944), Vervelde (1946) and Suneson *et al.* (1948) from comparisons between sister lines of crosses segregating in awn character or from

8B

experiments using backcrosses. The positive effect of awns has been mainly attributed to their ability to increase assimilation and transpiration and thereby the size of the grains.

The awn mutations obtained in N.P. 797, N.P. 798, N.P. 799 and N.P. 809 may hence be doubly advantageous, i.e. they help to satisfy the needs of the farmer and to enhance the yield of the strain. A study of the yielding ability of over 50 different awn mutations isolated in the four varieties already mentioned has shown that, though phenotypically identical, the awn mutants derived from the same strain differ widely in their performance in yield trials. This is probably conditioned both by the size of the chromosome segment deleted and by the changes effected in the other chromosomes of the complement. A further point worth recording is the occurrence of increased variability for polygenic traits in the selfed progenies of the awn mutants in comparison with similar progenies of the parent strain. This would suggest that there is much scope for recurrent selection in the progenies of such mutants for characters contributing to yield such as the number of ear-bearing tillers per plant, number of spikelets per inflorescence and the weight of grains. The induction of mutations and the conversion of a raw mutant into a marketable strain are not synchronous events and, for successful accomplishments in this field, it is essential that the breeder subjects the irradiated population to the selection and hybridization procedures developed in accordance with conventional genetic principles. Failure to recognize this fact has been largely responsible for the setting in of a sceptical and pessimistic outlook among breeders who took to mutation breeding with much excitement and empirical expectation.

Besides the fully awned mutants, the stiff strawed mutants (Fig. 4) found in all the varieties subjected to irradiation are of economic interest since lodging is a serious problem especially under irrigated conditions with possibilities for the application of fertilizers. These mutants differed widely in height, ear density and thickness of straw. All of them, however, had a reduced number of internodes, a short basal internode and a larger cross-sectional area particularly in the middle internodes when compared with the parent strains. In the M_2, M_3 and subsequent progenies of N.P. 797 treated with X-rays and P^{32}, mutants possessing resistance to additional races of black rust (*Puccinia graminis tritici*) and brown rust (*P. triticina*) were also isolated. The frequency and types of mutations isolated would largely depend upon the efficiency and direction of the selection pressure operated. Mutations affecting physiological and biochemical characters seem to occur fairly frequently but they need for their detection suitable techniques and, naturally, greater effort.

Of late, there has been increasing doubt about the possibility of adding a desirable trait to a variety through irradiation without at the same time

bringing about some detrimental changes. Our results with the awn mutations in varieties of bread wheat clearly suggest that at least in polyploid plants mutation breeding will be of value when the incorporation of a specific attribute to an already highly-bred strain is desired.

IV. Induced Mutations and Evolution of the Bread Wheat

(a) Genome sensitivity to radiations

In contrast to tetraploid emmer wheats, viable mutation frequency in hexaploid bread wheat is very high (Bhaskaran and Swaminathan 1960). A combination of the effects of duplicate factors at many loci and of functional diploidy in others seems to give rise to a high viable mutation rate in bread wheat. The restricted spectrum of the induced morphological mutations coupled with a high frequency of occurrence of each category of mutant would support this view. As between diploid einkorn wheat and bread wheat, the differential fitness of the latter to mutation breeding is explicable on the ground that polyploidy confers on bread wheat the advantage of viability in the face of even drastic intra- and inter-chromosomal changes induced by radiations. The reasons for the marked difference observed in the frequency of visible mutations occurring in emmer and bread wheats are, however, not clear. Using survival, frequency of aberrations per unit length of chromosome and M_2 chlorophyll mutation rate as indices of radiosensitivity, Pai and Swaminathan (1960) found that *A. squarrosa* is much more radiosensitive than *T. monococcum* and *A. speltoides* var. *ligustica*. Thus, it is probable that the *squarrosa* genome might have introduced into bread wheat a physiological state which promotes the incidence of a higher frequency of chromosomal aberrations and viable mutations following treatment with mutagens.

Emmer and bread wheats behave alike as regards survival in radiation experiments (Natarajan *et al.* 1958). Apparently during the evolution of bread wheat, further duplications for various genes concerned with basic metabolic processes have been lost. This would imply a considerable homogeneity with regard to such basic factors between the B and D genomes, thus lending further support to the hypothesis of Sarkar and Stebbins (1956) that the B genome in wheat is derived from a species of *Aegilops* and not from *Agropyron* as supposed earlier.

(b) Free-threshing nature and mutability

A comparative study of the induced mutation rate in the six commonly recognized hexaploid species revealed that the species which lack the 'Q'

factor (namely *T. spelta*, *T. macha* and *T. vavilovi*) had a significantly lower mutation frequency as compared to *T. aestivum*, *T. compactum* and *T. sphaerococcum* which all possess the '*Q*' factor and hence are free-threshing (Swaminathan and Rao 1960). The relatively greater mutability of the '*Q*' locus is also evident from the preponderance of speltoid mutations in *T. aestivum*.

(c) *Induced mutations and origin of bread wheat*

It is now known that *T. spelta*, *T. sphaerococcum* and *T. compactum* are each separated from *T. aestivum* by a single gene *Q* located on chromosome 5A, S on 3D and C on 2D respectively (Sears 1954). The number and location of the genes differentiating *T. macha* and *T. vavilovi* from *T. aestivum* have not yet been precisely determined, though there is evidence to suggest that only 1 or 2 genes may be involved in these cases also (Singh *et al.* 1957; Sachs 1953). MacKey (1954c) has hence suggested that all these species should be considered as sub-species of *T. aestivum*. MacKey (1954a) has further shown that *T. spelta* cannot be the primary hexaploid wheat and has suggested that it might have arisen from the cross *T. compactum* (*antiquorum*) ×*T. dicoccum*. Mutation experiments carried out at the Indian Agricultural Research Institute in all the six hexaploid *Triticum* species show that while *aestivum* (*sensu stricto*) forms can be obtained from *T. compactum*, *T. sphaerococcum*, *T. macha* and *T. vavilovi*, such types never arise from *T. spelta*. This would lend further support to the view that *T. spelta* is not the progenitor of *T. aestivum*. It seems likely to us that the hexaploid *Triticum* species are derived from two independent hybridizations followed by chromosome doubling. First, *T. spelta* could have arisen in the way suggested by Kihara (1944) and McFadden and Sears (1946), i.e. through amphidiploidy in crosses between *T. dicoccoides* or *T. durum* or *T. turgidum* and *A. squarrosa*. Secondly, *T. aestivum* might have originated from a cross between *T. persicum* (a tetraploid wheat with '*Q*' factor) and *A. squarrosa*. This view is in consonance with the induced mutation data and also receives support from the fact that *T. persicum* and *A. squarrosa* occur in the same region, which is also the gene centre of *T. aestivum* (Vavilov 1923; Zhukovsky 1923; MacKey 1954a). Another point of interest emerging from the mutation data is that *T. sphaerococcum* could not have arisen through a deletion from *T. aestivum* as suggested by Ellerton (1939), since *aestivum* like mutants occur frequently in the irradiated progenies of *T. sphaerococcum*. The S gene, which behaves as a complex locus in mutation experiment, is similar to a recessive epistatic gene in effect. Mutation analysis, besides being of considerable interest to the wheat breeder, is thus very helpful in tracing evolutionary trends among wheat species.

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LEGENDS TO FIGURES (PLATES IX, X AND XI)

FIG. 1. Autoradiograph of wheat seedlings raised from seeds treated with P³². The seedlings showing stronger radioactivity are from 10 μc per seed treatment and the others are from 5 μc seed treatment. This method of treatment is very effective in inducing mutations in wheat.

Fig. 2. Ears of the wheat variety N.P. 809. This variety has resistance to several races of black, brown and yellow rusts but the ears lack awns.

Fig. 3. Ears of an awned mutant of N.P. 809 isolated in material treated with P^{32}.

Fig. 4. A true breeding progeny of a short and stiff strawed mutant of the variety N.P. 797 growing along with the mother strain.

A REVIEW ON
I. PREVALENCE OF CANCER IN INDIA
II. CANCER OF THE CERVIX (INCIDENCE, RADIOBIOLOGY, SPECIAL SURGICAL TECHNIQUE AND EVALUATION OF RESULTS)

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ABSTRACT

Difficulties of determining the incidence and prevalence of a disease in a vast and under-developed country like India have been discussed. Previous work on cancer mortality has been assessed. In the new assessment, prevalence has broadly been equated to fatality to cancer. Probabilities of deaths from cancer at various ages have been worked out. Data for Calcutta Corporation area and that of the U.S.A. have been compared. A dimensional estimate based on Calcutta data has been attempted to find the extent of prevalence of cancer in India. Basic assumptions are explained and a mathematical relationship between incidence and prevalence given. Prevalence of cancer in India is estimated at 1·8 millions.

Comparative incidence of cancer in different sites of Chittaranjan Cancer Hospital, Calcutta, and Royal Cancer Hospital, London, has been shown. Ethnological variations are discussed. Reasons for taking up surgical treatment in addition to radiotherapy are given. As radio-reaction of cancer cells is unpredictable, selection of cases for surgical treatment was based on operability. Mitra technique for cancer of the cervix is presented with its five-year end-results. Five-year salvage percentage in cancer positive lymph nodes is given.

A preliminary note to assess the effect of Au^{198} in cancer of the cervix is appended.

The investigators in medico-biological sciences have taken up the challenge to fight cancer with knowledge. As it stands today, except in un-developed tropical countries, cancer is No. 2 killer of human beings, No. 1 being heart disease.

During the last 50 years, extensive and exhaustive investigations have been carried on from clinical and demographic points of view, but fundamental researches through biological studies have been taken up not earlier than a quarter of a century ago. Ideas are born in individual minds but their formulation is influenced by a wide variety of contacts and their translation into practical reality is aided by the entire scientific community.

I. PREVALENCE OF CANCER IN INDIA

Assessment of the prevalence of a disease is not an easy task, specially for a vast country like India, having a population of nearly four hundred millions extending over 1·2 million square miles. Excepting a few principal

cities, a greater part of the land is underdeveloped and the registration for vital statistics inadequate. Proper assessment is hardly possible without sampling studies of the whole population.

The incidence of cancer is usually deduced from hospital records by way of admissions and autopsies, as well as from total mortality figures in general. Clinical assessment is often vitiated by wide errors unless checked by biopsies which are not always possible. Autopsies will perhaps give a more correct information, but autopsies for 100 per cent deaths can never be possible. Even in a well-organized country like Germany, where the post-mortem examination was obligatory in all the State hospitals, autopsies were made on an average of 4·3 per cent of the total deaths of the land (Lubarsch 1918).

Certain amount of work on cancer mortality in India has already been done. From the initial subjective opinion that, as compared to the European races, the Indian races were less susceptible to carcinoma, there has been a gradual shift; analysis of hospital and autopsy records and the reported causes of death in city population tend to show that cancer mortality is not much lower in India than in the West. But the picture is still far from definite.

Some investigations in the line were made by the author (Mitra and Das Gupta 1959) with the data from the city of Calcutta. The prevalence has been broadly equated to fatality to cancer. Before dealing with the death records of Calcutta Corporation area (on which this note mainly relies), a passing mention may as well be made of other relevant data collected for this purpose. For example, out of total 9,130 deaths occurring among the in-patients of the Calcutta Medical College Hospital during 1946–50, only 249 deaths (or 2·7 per cent) were caused by cancer; the annual rate of cancer deaths in the hospital actually varied between 2·2 per cent (1942) and 3·8 per cent (1943) during this period. Autopsy records of the same hospital for the same period disclosed only 11 cancer deaths out of 549 total cases, giving about 2 per cent cancer mortality. Autopsy records of Rogers (1925) from the same hospital showed 4·59 per cent cancer mortality. Data were also collected from a few insurance companies in India and the cancer mortality was seen to vary between 2 and 3·5 per cent among the assured lives. These total measures of mortality are, however, of little validity or use for comparative study; they are, besides, based on highly select and rather scanty material. Nath and Grewal from collected autopsy records of different parts of India and Burma found cancer mortality rate at 4·2 per cent. Khanolkar (1945) had 4·41 per cent from the Bombay K.E.M. Hospital autopsy records. The percentages given by Nath and Khanolkar are to be considered as *relative* cancer mortality figures because they eliminated deaths due to infectious and tropical diseases from the total number of deaths.

To enable better assessment and comparisons, the total deaths registered with Calcutta Corporation were examined in detail. The proportion of uncertified deaths was very low; less than 2 per cent of total deaths in Calcutta, whereas 98 per cent of deaths were certified by qualified medical officers.

Out of total certified deaths of 64·487 (i.e. 32,113 in 1954 and 32,374 in 1955), the proportion of cancer deaths came out as 2·4 and 2·8 per cent respectively. The variation between the years appeared to be high for a cause like cancer, but improved identification was probably responsible for the increased proportion in 1955. While the proportion of cancer deaths to all deaths generally rose to a maximum in the 50–60 age-group and declined after that, cancer mortality as such was substantially higher in the ultimate 60–above age-group.

The information about probabilities of deaths from cancer at various ages will be interesting. The probabilities found by fusing 1951 West Bengal Census Life Table mortality with Calcutta cancer death proportions in age-groups have been worked out. People at large will be more interested in knowing his own chances of escaping or dying of cancer than in the mean annual mortality from it. The probability of death from cancer was of the level of 2·8 per cent at birth, and this rose to level of 6·8 per cent by the time age 40 is attained. The probability of death from cancer was higher for the female than for the male up to age 40; the position got reversed by age 60.

The percentage distribution of cancer deaths by age for the Corporation area was compared with similar distribution for the U.S.A. data given by Steiner (1954) : The comparison is presented graphically in Fig. 1 for males. The age distribution patterns of cancer showed good agreement between the two countries excepting at the very advanced age. The age distribution of cancer of the female genital organs was also similarly compared (Fig. 2) : The patterns of age distribution of this particular site disclosed strikingly close agreement. Assuming the defects in diagnosing and reporting which pulled down the total, the patterns could have such close resemblance.

An Estimate of the Prevalence of Cancer in India

Since the extent of prevalence of cancer in India was not known, a dimensional estimate based on the Calcutta investigation is attempted below. A number of broad assumptions had to be made and the estimate is somewhat speculative. Yet it is clear that, in view of the grave defect in the sphere of diagnosing and reporting, even a very rough and tentative estimate indicating the floor level of prevalence will be useful as a starting point.

The first important assumption was that the Calcutta Corporation cancer death proportions could be taken as representative of the country as a whole. Other assumptions were about the current level of total mortality in the country and the distribution of life with cancer over duration since onset.
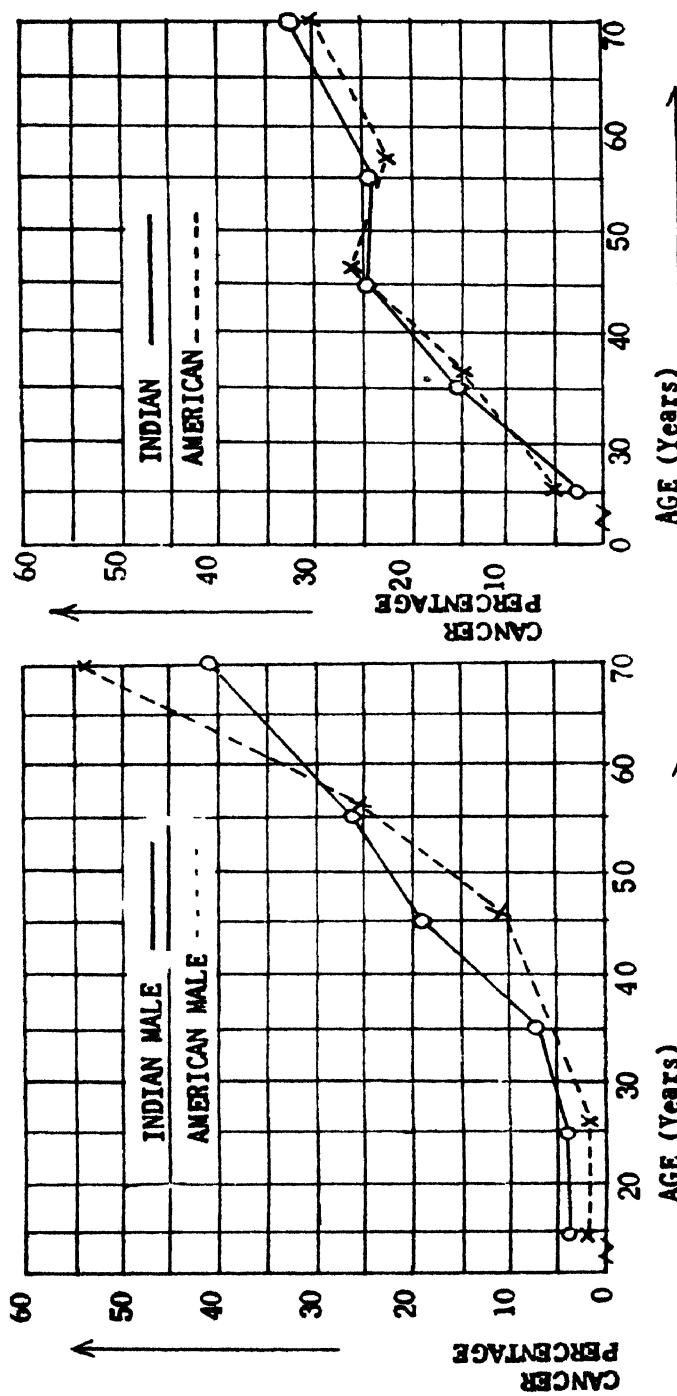
FIG. 1. Percentage distribution of cancer deaths by age.

FIG. 2. Percentage distribution of deaths with cancer of female genital organs by age.

(Calcutta Corporation records 1954 and 1955. Steiner 1954)

In any region, the community habits are not basically different between the rural and the major bulk of the urban sector; nor are any other sharp differences between the rural and the urban environmental factors known which might introduce a significant disparity in carcinogenic dosage, except perhaps in relation to cancer of the lung. Lung cancer is of very minor frequency even for Calcutta Corporation area. The Calcutta experience could be therefore representative of the country in this respect, particularly as it is a big cosmopolitan city inhabited by people from many parts of India, and the migrants usually carry their habit patterns with them.

But owing to the location of specialized cancer institutions and hospital departments, and because of the availability of advanced surgical facilities, generally more cancer cases come to Calcutta; the question therefore was whether this eventuality resulted in a higher proportion of cancer deaths in Calcutta Corporation area. Few cases died while on the operation table or while being treated actively and not in removal state. With no hope for recovery, people prefer to return to their own home surroundings in the last days, specially in an affliction like cancer when there will be nothing more to be done. General reasoning therefore warrants the assumption that the Calcutta Corporation deaths will not be inflated by migrated cancer cases, and informed clinical opinion is not to the contrary. We therefore consider the assumption valid that the cancer death proportions of the Corporation area hold generally for the country.

Adopting the current national death rate as 20 per thousand broadly, the total annual deaths from all causes will number 7·6 millions in the estimated 1955 Indian population of 380 millions ; applying the Corporation cancer death proportions, the estimated cancer deaths number 0·198 or 0·2 million roughly.

The next problem was to relate the cancer death proportion to the prevalence rate. Under stationary conditions, the rate of incidence will be equal to the death rate plus the cure rate. The cure rate in the case of cancer being small for the country as a whole, the cancer death rate may be taken as the rate of cancer incidence for the purpose of this rough approximation; the rate of incidence will actually be slightly higher.

The relationship between the rate of incidence and the rate of prevalence could be discussed with advantage on a theoretical plane at this stage. If i be the annual rate of incidence and $f(t)$ the frequency distribution of cases living with cancer over duration t since onset, so that $f(o) = 1$ and $f(\infty) = 0$, then P the rate of prevalence is given under stationary condition by the area integral :

$$P = i \int_0^\infty f(t). \, dt.$$

While the mathematical relationship between incidence and prevalence given above is quite straightforward, all its implications are not. For example, if cancer of different organs were considered separately, it will be found that the organ prevalence rates will not be proportional to the relative incidence rates; but will differ according to the average duration of life with cancer for the particular organs.

The duration of life with cancer varies by sites and types of cancer. But it is not possible to introduce any refinement for this variation in the present attempt at first approximation. The average duration of life with cancer even for all cancers taken together is not known firmly for any country, much less for India. A broad assumption was again made on the basis of our experience; and the shape of frequency distribution of life with cancer was taken as in Fig. 3; if such a distribution holds, the average duration of life with cancer is about three years, and the prevalence of cancer in India amounts to 0·6 million in round figures.
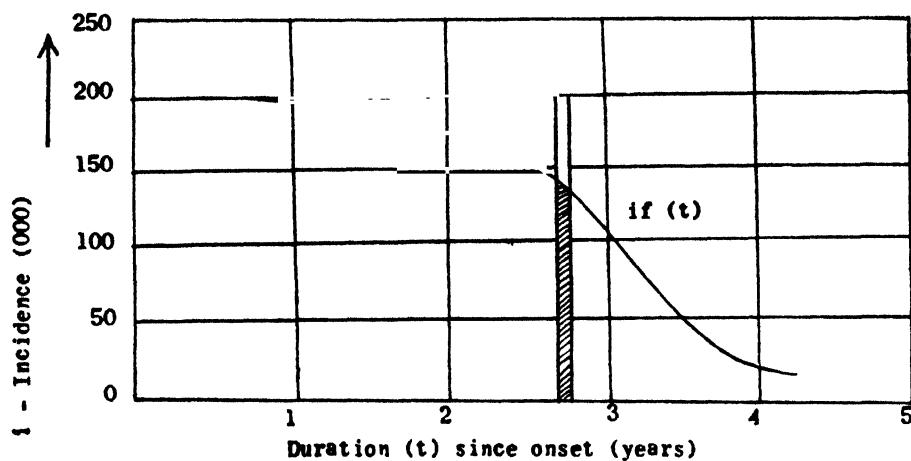
FIG. 3. Frequency distribution of the living with cancer (author's experience).

Personally I feel that the gaps in diagnosis and in reporting are very substantial and the prevalence of cancer in India may be at least three times as much as has been estimated, i.e. 1·8 millions. This statement may be substantiated by the fact that though it is not definite that vulnerability to cancer is the same in India as in, say, U.K., age-specific Calcutta reported cancer death rates applied to U.K. age structure produce an overall cancer death proportion of 7·6 per cent (of total deaths), whereas the actual reported proportion in U.K. (1953-54) is 17·9 per cent. On the other hand, age-specific cancer death rates of U.K. (1953-54) applied to the Calcutta age structure produced an overall cancer death proportion of 6·5 per cent instead of 2·6 per cent as has

been actually registered in 1954 to 1955. Hence my assumption that the prevalence of cancer in India is at least 1·8 millions.

II. CANCER OF THE CERVIX

(INCIDENCE, RADIOBIOLOGY, SPECIAL SURGICAL TECHNIQUE AND EVALUATION OF RESULTS)

Although the incidence of cancer does not vary much in different countries of the world, it has been well established that the anatomical distribution of cancer is not the same everywhere.   The following percentage distribution diagram (Fig. 4) shows the comparative incidence of cancer in different sites of two institutions, namely the Chittaranjan Cancer Hospital of Calcutta and the Royal Cancer Hospital of London.
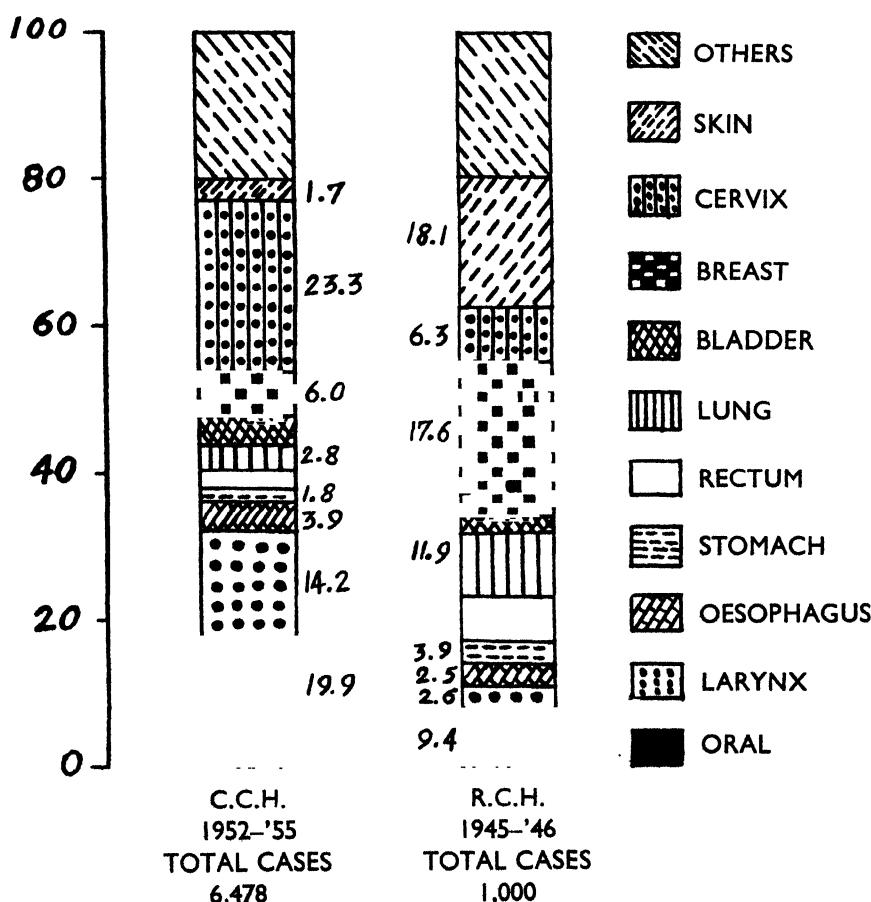
FIG. 4.   Comparative incidence of cancer in various sites : the Chittaranjan Cancer Hospital of Calcutta, India, and the Royal Cancer Hospital, London.

The preponderance of oral, laryngeal and cervical cancer is very definitely marked in India. During the period of five years from 1954 to 1958, 4,258 female malignant cases were detected having cancer in different parts of the body, of which 483 (11·3 per cent) were cancer of the breast, 2,420 (56·8 per cent) of the female genitalia, 2,135 (50·1 per cent) of the cervix and only 61 (1·4 per cent) of the body of the uterus. Thus the ratio is : for each cancer of the breast, there are 4 cancer of the cervix, and for each cancer of the body of the uterus, there are 35 cases of cancer of the cervix.

Cancer of the cervix was believed to be rare among the Mohammedan women but the experience of the author is otherwise. The comparative incidence of cancer of the cervix between the Hindus and the Mohammedans has been worked out by the author. Tables I and II indicate that the incidence is practically the same between the two communities, the difference noted not being statistically significant. This leads us to presume that it is not the ethnological difference but the habits and environments, social status and assaults on the cervix due to repeated childbirths which are responsible for the frequency of cancer of the cervix in one or the other community.

TABLE 1

Incidence of cervical cancer on ethnological basis : Chittaranjan Seva Sadan, College of Obstetrics, Gynaecology and Child Health, Calcutta

Religious group	1940–1950		
	Total number of gynaecological cases	Cancer cervix cases	Percentages*
Hindu	89,271	569	0·637 ± 0·03
Muslim	4,556	20	0·439 ± 0·10
Others	550	4	
Total	94,377	593	

* Difference between the Hindu and Muslim is not statistically significant, the value being 0·198 ± 0·102.

Recently there is a great move all over the world to screen the adult women free from any suggestive symptoms of malignancy with a view to finding out cases of early cancer amongst the unsuspected people. We have started similar type of work here at the Chittaranjan Seva Sadan and Chittaranjan Cancer Hospital.

Three thousand four hundred and fifty-eight women were screened of which 2,966 having some gynaecological symptoms not suggestive of malignancy ; 149 without any symptoms ; 316 and 27 being in the early and late months of gestation respectively.

TABLE II

Incidence of cervical cancer on ethnological basis : Chittaranjan Cancer Hospital, Calcutta
(1950–1955)

Year	Total gynaecological cases attended			Cancer cervix detected		
	Hindu	Muslim	Others	Hindu	Muslim	Others
1950	487	8	5	288 (59·1)	4 (50·0)	3
1951	755	19	9	396 (52·4)	10 (52·6)	3
1952	797	25	12	379 (47·6)	16 (64·0)	7
1953	747	31	10	328 (43·9)	15 (48·4)	2
1954	794	37	9	341 (42·9)	25 (67·5)	3
1955	908	56	15	369 (40·6)	32 (57·1)	11
Total	4,488	176	60	2,101 (46·8 ± 0·74)	102 (57·9 ± 3·72)	29

Difference between the Hindu and Muslim is statistically significant at 5 per cent level, the value being 11·1 ± 3·79.

Altogether 9,044 smears were taken —6,916 being primary and the rest repeat.

Cancer of the cervix was detected by smear technique and confirmed by biopsy in 18 cases having a prevalence rate of 0·52 per cent—12 of them being invasive and the rest intra-epithelial. If only the invasive cases are taken into consideration, the prevalence rate becomes 0·34 per cent. This percentage meets with the general agreement of other workers in different parts of the globe.

I have already pointed out that cancer of the body of the uterus is very rare in my series, the ratio between cancer of the body of the uterus to that of the cervix being 1 : 35. So far as my information goes, cancer of the body of the uterus is a rare thing all over India, although in the Western countries and even in Japan the incidence of cancer of the body of the uterus is much higher.

In order to ascertain the incidence of cancer of the body of the uterus amongst the unsuspected group of adult females above the age 35, 2,037 patients from the polytechnic of the Chittaranjan Seva Sadan were screened by taking smear as well as intrauterine aspiration smear. Altogether 6,394

smears were taken including repeat examinations. Smear was positive in 10 cases and endometrial biopsy proved cancer of the body of the uterus in 9, thus giving a prevalence rate of 0·44 per cent. It has been noted previously that screening results of unsuspected group for cancer of the cervix gave us a prevalence rate of 0·34 per cent, showing thereby that amongst the unsuspected group with minimal symptoms the prevalence rate of cancer of the cervix and cancer of the body of the uterus is almost the same. It will have to be worked out why, at a later stage, cases of cancer of the body of the uterus do not present themselves for treatment. It might be that the growth of the cancer in body of the uterus is less rapid than in the cervix and before the growth is well established, a good number of these patients die of some other disease.

While I submitted my 5-year statistical end-results of cancer of the cervix treated with radiation therapy in the Third International Radiological Congress in Paris in 1931, I was very much impressed to find continental surgeons performing the radical vaginal hysterectomies in similar cases.

Radiation therapy has already been accepted by us but the surgical treatment has been reintroduced and is gaining popularity because of the limitations and hazards of radiation therapy.

It is a moot question why I took up the surgical treatment in addition to radiation therapy. To begin with, I used to treat all cases with radiotherapy as was usually the custom during the earlier part of this century. My first 5-year end-results, though not very encouraging, was presented at the Third International Radiological Congress. It was 16·6 per cent (Mitra, 1932). Since then I took up surgery along with radiotherapy for the treatment of cancer of the cervix. The results of radiotherapy improved no doubt in course of time and with the addition of supervolt therapy. But the results of surgical treatment were definitely better than radiotherapy. Table III shows the comparative 5-year end-results of Stages I and II cases during the years 1950 and 1951. They are 47·4 per cent and 65·6 per cent for radiotherapy and surgery respectively.

TABLE III

Cancer of the cervix—Stages I and II : comparative 5-year end-results

Year	Radiotherapy		Surgical treatment	
	Total number treated	Cure rate (per cent)	Total number treated	Cure rate (per cent)
1950 and 1951	78	47·4	32	65·6

I have not yet been in a position to select my cases on the basis of radio-resistance and radiation response. We have tried to assess the radiation response of cervical cancer cases by studying the reaction of different types of cells (resting, mitotic, differentiating and degenerating) from 'young tumour foci' after the first radiation exposure (7,000 r). Our results do not correspond with those of Glücksmann (1941). In our series, the radiation response has been found much the same in all cases at the end of the first week after radiation. The percentage count showed an increase in the proportion of degenerating and differentiating cells and a corresponding decrease in the proportion of mitotic and resting cells uniformly in all cases. Hence we could not predict radio-resistance after initial radiation as has been suggested by Glücksmann (1941).

Heymann (1950) does not believe that any group of cervical cancer is specially radio-resistant but he considers that some tumours are more malignant than others. This varying degree of malignancy is detected from clinical experience and cannot be substantiated by microscopical evidence. Although valuable contributions have been made by Glücksmann and Ruth Graham in this direction, the real solution to unfold the varying degree of malignancy is yet to be sought for.

Under the circumstances, the selection of my cases for surgical treatment was based on whether a case can be operated or not. I was not particular in selecting a model series of thin, comparatively young women with an ideal picture of health and having a small circumscribed growth in the cervix. My cases were recruited both from thin and obese patients, and from Stages I, II and III groups. Malnutrition was rather the prevalent feature in most of my cases. Only those cases were taken up who willingly submitted themselves for operation, the remaining cases were treated by radiotherapy.

In 1932 I started the surgical treatment in addition to radiation therapy. Prior to this period, Wertheim's radical abdominal operation was tried with an enormous primary mortality. This was partly due to devitalized conditions of patients undergoing operation. Anaemia, low blood volume, and a hyposthenic condition were usual in such cases. So, instead of taking up the radical abdominal operation, I preferred radical vaginal method. Although antibiotics and sulfa drugs were not introduced at the earlier part and blood for transfusion was not easily available, I was much impressed with a remarkably low primary mortality (Mitra 1939). The primary mortality in the last 105 cases of the total 209 cases, operated by me during the period 1932 to 1950, was nil (Table IV), although taking all the cases together it was 2·8 per cent (Mitra 1955).

During the last 10 years, a total number of 216 cases of cancer of the cervix were operated by the new technique although the total number of radical vaginal hysterectomies so far done here and abroad amounts to 450.

TABLE IV

Total number of operations done by Mitra during
the period from 1932 to 1950*

Number of cases operated	Operability rate (per cent)	Primary mortality (per cent)
209	45·8 (17)	2·8
Last 105 cases		0

* Subodh Mitra (Chittaranjan Seva Sadan and Cancer Hospital).

Out of 216 cases dealt with by the new technique, 194 cases were operated by the author and the rest by his colleagues and assistants. When classified stage by stage, 114 belonged to Stage I, 80 to Stage II and 22 to Stage III (Table VI).

The *primary mortality* of my new series was 3·6 per cent. Unfortunately, we had a few accidental deaths due to malignant tertian malaria, haemolysis by blood transfusion and opium-poisoning. If these were excluded, the corrected mortality becomes 1 per cent. There was no primary mortality for the last 55 cases.

Post-operative bladder troubles were remarkably few; the residual urine being reduced to about 2 oz. within an average of 10–12 days.

There was no ureteric fistula in this series. Two patients had vesico-vaginal fistulae, both having had previous radium treatment in another hospital.

Associated pelvic lesions of the nature of tubo-ovarian masses, ovarian or pelvic endometriosis or uterine tumours are said to cause difficulties in radical vaginal operations. Out of 450 radical vaginal hysterectomies done by me including the new series, tubo-ovarian masses were encountered in 18 cases, ovarian endometriosis in 5, uterine fibroids in 7, pyometra in 13 and uterine pregnancy in 4. Table V gives the detailed list of associated pelvic lesions.

Pelvic lymph nodes are removed, as far as practicable, mostly by the extra-peritoneal route and some (paraureteric nodes) by the vaginal route. It is not possible to remove all the lymph nodes, big or small, from the pelvis. That is why, perhaps, Wertheim used to remove only the palpable nodes and Schauta did not attempt to remove any. Amreich (1955), quoting a study of pelvic nodes by Riffenstuhl, has shown that there are at least two ungettable groups of glands in connection with the cervix, namely (a) glands situated at the most lateral part of the Mackenrodt's ligament, the connecting lymph vessels accompanying the inferior gluteral artery and the pudeldal vessels, and (b)

TABLE V

Pelvic lesions associated with cervical cancer cases operated

Total number of operations	Associated pelvic lesions		
Radical vaginal hysterectomies (new series included) 450	Multiple fibroids 7		
	Tubo-ovarian masses 18		
	Ovarian endometriosis 5		
	Hydrosalpinx 3		
	Pyometra 13		
	Dermoid cyst 1		
	Ovarian cyst 1		
	Other conditions 7		
	Pregnancy 4		

pararectal glands which remain under the rectal fascia and whose lymph channels accompany the haemorrhoidal vessels. These two groups cannot possibly be removed by any operation, nor can they be effectively sterilized by radiation. 1 had difficulties in two cases, each belonging to Stage I. In both these cases, none of the glands removed showed any metastatic cancer cells; but within six months after the radical operation, a nodular growth was found in each case in the pelvis in the neighbourhood of the ischial spine. This might be from one of the ungettable groups of lymph nodes cited by Amreich.

Systematic removal of all the detectable lymph nodes was done in each of the whole series of 216 cases. Table VI gives the incidence of cancer positive nodes. In 22·6 per cent of the total number of cases, the positive nodes were detected, which breaks up into 15·8 per cent in Stage I, 27·5 per cent in Stage II and 41·0 per cent in Stage III.

TABLE VI

Incidence of cancer positive nodes

Stages	Number of cases	Cancer positive glands	Percentage
I, II and III	216	49	22·6
I	114	18	15·8
II	80	22	27·5
III	22	9	41·0

Further analysis shows that the internal iliac and obturator nodes were most commonly affected. Recently I have started removing lower lumbar group of glands (paraaortic) by extending upwards the left extraperitoneal

incision. No evidence of metastasis of this paraaortic group of glands was found in 25 cases so far operated except in two cases belonging one to Stage III and another to Stage II. The latter case, although it belonged to the clinical Stage II, had 9 positive nodes out of 13 glands removed.

Parametrial infiltration.—It has been found by serial sections of the parametrium that only in 32 out of 216 cases the parametria were infiltrated with cancer cells either in the proximal, middle or in the distal third from the uterine side. Thus, parametria were found infiltrated with cancer cells in 14·7 per cent of the total number of cases operated, while the nodal involvement was found in 22·6 per cent in the same series (Table VII). Further analysis of our data indicates that in only 14 cases, both the parametrial and the pelvic nodes were infiltrated simultaneously. This shows that, whatever may be the clinical staging, one is not justified in limiting the surgery in any stage of cancer of the cervix except, of course, in Stage 0 which has not been included in this series.

TABLE VII

Parametrium infiltration and pelvic node metastasis

Number of cases operated	Parametrium infiltrated (per cen')	Pelvic node metastasis (per cent)
216	32 (14·7)	49 (22·6)

EVALUATION OF 5-YEAR END-RESULTS

The acid test of a new technique is shown not only by the extensive and successful removal of the cancer-bearing tissues but also by the evaluation of 5-year end-results. The materials at hand are not large but may be taken to be sufficiently representative. During the years 1949 to 1953, 57 cases belonging to Stages I, II and III were operated by the new technique, yielding

TABLE VIII

Five-year end-results of cancer of the cervix treated surgically by Mitra technique

Years under review	Total number	Stages	Five-year salvage Number	Five-year salvage Percentage	L.S.O.* (per cent)
1949-1953	57	I, II and III	35	61·4	5·5
	38	I	25	65·9 ⎫ 64	
	12	II	7	58·3 ⎭	
	7	III	3	42·8	

* L.S.O. indicates lost sight of cases.

an overall 5-year cure rate of 61·4 per cent. By breaking down the results, 64 per cent has been salvaged from Stages I and II cases (Table VIII). Stage I results of 1952 have been vitiated by a few accidental deaths by malignant tertian malaria, opium-poisoning and haemolysis after blood transfusion.

Five-year end-results were satisfactory in the first series covering the period 1949 to 1951, yielding 75 per cent 5-year cure in Stages I and II (Table IX).

TABLE IX

Five-year end-results of cancer of the cervix treated surgically by Mitra technique

Years under review	Total number	Stages	Five-year salvage	
			Number	Percentage
1949–1951	20	I, II and III	14	70
	16	I and II	12	75

Comparing the results of the present series treated by the new technique with those of my previous cases treated by Schauta's operation, it can be seen that the results of the present series (61·4 per cent) are better than those of Schauta's series (44·5 per cent) (Table X).

TABLE X

A comparative 5-year end-results of cancer of the cervix treated by Mitra technique and by the Schauta operation

Years under review	Type of operation	Total number and stages	Five-year salvage		L.S.O. (per cent)
			Number	Percentage	
1943–1951	Schauta technique	74 I, II and III	33	44·5	
1949–1953	Mitra technique	57 I, II and III	35	61·4	5·5

Five-year end-results have also been calculated on the basis of cancer positive and cancer negative pelvic nodes of the new series. There was 73·8 per cent 5-year cure rate in gland negative cases against 26 per cent in gland positive ones (Table XI). In the previous series of 1949–51, 5-year salvage of gland negative cases was 84·6 per cent. Unfortunately, the percentage of gland negative cases has been vitiated by a few accidental deaths in 1952. Prognosis in gland metastasis cases is unfavourable. But the result that, even in cases with metastasis, 26 per cent had 5-year cure

when regional lymph nodes were sufficiently excised, encourages the surgeons and shows the importance of lymphadenectomy.

TABLE XI

Five-year end-results in cancer positive lymph nodes

Years under review	Type of operation	Pelvic nodes					
		Negative			Positive		
		Total number	Five-year salvage		Total number	Five-year salvage	
			Number	Percentage		Number	Percentage
1949–1953	Mitra technique	42	31	73·8	15	4	26

Supervolt radiotherapy is given as a routine treatment to those cases where there is parametrial or nodal metastasis.

CONCLUSION

The rational conclusion I can draw from my experience is that this new operation satisfies all the essential conditions for the radical surgery of cancer of the cervix. This operation can be done with a good amount of safety even in the aged, comparatively bad surgical risk cases and in heavy corpulent patients. Besides, there is an additional advantage of the rehabilitation of the prolapsed bladder after the extensive removal of the vagina. There are fewer post operative complications, both immediate and remote. There is no ureteric fistula, and bladder troubles are minimal. The end-results are satisfactory.

Stump carcinoma, vaginal carcinoma and cancer of the body of the uterus can be electively operated by this new technique.

A PRELIMINARY NOTE TO ASSESS THE EFFECT OF AU[198] IN CARCINOMA OF THE CERVIX

On an experimental basis, we took up a series of 12 cases of cancer of the cervix for pretreatment with radioactive gold (Au[198]), seven to ten days before the radical operation. About fifteen millicuries of Au[198] were injected to each parametrium with a view to studying its effect on the pelvic nodes. During the operation, not only the nodes but also the parametrium, the uterus, the uterine tubes, the ovaries, the base of the bladder, and in some

cases even the ureter were found tinted.   The whole field of operation, both extraperitoneal and vaginal, was congested causing a great deal of haemorrhage. Average quantities of radiation received in individual nodes are as follows : common iliac node—2,610 rep., external iliac node—2,700 rep., internal iliac node—5,210 rep., and obturator node—3,500 rep.

This experiment neither facilitates clean dissection of nodes, nor can it be helpful in sterilizing cancer cells from inaccessible small nodes with the small amount of radiation received.   It is not also advisable to increase the dose of Au[198] for fear of increased reaction and further congestion of neighbouring tissues.

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GENUS *CHILOSCYPHUS* (CORDA) SCHIFFNER AND *HETERO-SCYPHUS* SCHIFFNER IN INDIA—I

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ABSTRACT

The genus *Chiloscyphus*, with a cosmopolitan distribution, is well known through its widely distributed species, *C. polyanthus* (L.) Corda. Stephani (1906–1909, 1917–1924) recognized 175 species in this genus and 11 of these, viz. *C. argutus, C. coalitus, C. perfoliatus, C. flaccidus, C. inflatus, C. gollani, C. himalayensis, C. communis, C. gammianus, C. polyanthus* and *C. campanulatus*, occur in India (Mitten 1860; Stephani 1906–1909, 1917–1924; Kashyap 1932; Chopra 1943). From a critical analysis of *Chiloscyphus* Corda, Schiffner (1910) recognized two distinct taxa:—

(1) with *intercalary androecia* on main stem or on side branches and with perigonial leaves largely similar to vegetative leaves, and

(2) with *terminal group of androecia* arranged spicately and borne on branches arising laterally from the angles of amphigastria and with perigonial leaves different from sterile leaves.

The former has been retained by him (Schiffner 1910) under the old genus *Chiloscyphus* (Corda) *emend.* Schffn., while the latter was transferred to a new genus *Heteroscyphus* Schffn. Schiffner's new genus has been recognized by several hepaticologists (Verdoorn 1932; Buch, Evans and Verdoorn 1938; Evans 1939). Judged from the concept advanced by Schiffner (1910), four of our species, viz. *C. argutus, C. coalitus, C. communis* and *C. perfoliatus*, belong to *Heteroscyphus* and the rest to *Chiloscyphus* except *C. inflatus* which, in character of cells in the leaf and the amphigastrium and in its overall appearance, is significantly different from either *Heteroscyphus* or *Chiloscyphus*.

The present communication is based on 'PANDÉ COLLECTION' from specimens collected by Decoly and Schaul from Darjeeling and its neighbourhood, and sent to one of us (Pandé) by the late Prof. V. Schiffner, Pfleiderer's collection of the Western Ghats, Fr. Foreau's collection from South India and our own collections from several parts of the country. A key for the identification of all the species known from India along with a detailed illustrated account of *C. polyanthus, C. campanulatus, H. argutus* and *H. coalitus* is included in this paper.

INTRODUCTION

The genus *Chiloscyphus* Corda is an interesting member of the Acrogynous Jungermanniales with more or less cosmopolitan distribution, being known from nearly all parts of the world, particularly through its widely distributed species, *C. polyanthus* (L.) Corda. In his comprehensive treatment of the genus, Stephani (1906–1909) recognized 137 species, based primarily on their geographical distribution, and segregated them on the character of the leaves under three groups:

(a) *Integrifoliae*—species characterized by entire leaves,

(b) *Bidentes*—species with two teeth on the leaf margin, and

(c) *Pleuridentates*—species with several teeth on the leaf margin.

Nearly four years later Schiffner (1910), in a critical evaluation of the generic concepts of *Chiloscyphus*, stressed the fact that it is a heterogeneous assemblage of plants which, though largely similar in the character of the fertile branches and perianth, are entirely different in their total appearance particularly so in the feature of the androecia. He segregated these plants into two distinct taxa, (i) with *intercalary androecia* on the main stem or on side branches and with perigonial leaves, though distinct in their saccate appearance and a toothed dorsal flap, largely similar in size and shape to the sterile leaves, and (ii) with *terminal group of androecia arranged spicately and borne on branches arising laterally from the angles of amphigastria* and with perigonial leaves smaller in size and thus different from sterile leaves.

Schiffner (1910) retained the former to represent the genus *Chiloscyphus* Corda *emend*. Schffn., while the latter was considered to be sufficiently distinct to warrant its segregation into a new genus which he (Schiffner) designated as *Heteroscyphus* Schffn.

In a later publication Stephani (1917–1924) enumerated 38 additional species of *Chiloscyphus* without assigning them to the groups earlier proposed by him and also without recognizing the then newly advanced concept of the genus by Schiffner (1910). In all Stephani (1906–1909, 1917–1924) recognized 175 species belonging to this genus.

The earliest record of the genus *Chiloscyphus* from India is apparently from the publication of Mitten (1860) who listed three species, viz. *C. argutus* Nees (5–6,000 ft.), *C. coalitus* Hook. (4–6,000 ft.) from Khasi hills (Assam) and *C. perfoliatus* Mont. from the Nilgiris. According to Stephani (1906–1909, 1917–1924), seven species of this genus occur in India, six of these, viz. *C. inflatus* St. (Himalayas), *C. gollani* St. (Mussoorie), *C. himalayensis* (Mussoorie), *C. flaccidus* St. (Himalayas), *C. communis* St. (Assam) and *C. gammianus* St. (Himalayas), are new species instituted by Stephani while *C. perfoliatus* was earlier recorded by Mitten (1860). Kashyap (1932) collected five species, viz. *C. inflatus*, *C. argutus*, *C. himalayensis*, *C. polyanthus* and *C. campanulatus*, and along with these included the description of another species *C. gollani*. Thus, as has already been listed by Chopra (1943), 11 species are so far known from India: *C. argutus*, *C. coalitus*, *C. perfoliatus*, *C. flaccidus*, *C. inflatus*, *C. gollani*, *C. himalayensis*, *C. communis*, *C. gammianus*, *C. polyanthus* and *C. campanulatus*.

A perusal of the literature on the genus *Chiloscyphus* in India would indicate that our knowledge is still fragmentary and inadequate and treatment in the past lacks details of taxonomic features and suitable illustrations.

Stephani (1906–1909, 1917–1924) undoubtedly gave the latin diagnosis of the species treated by him but his account lacks illustrations. Kashyap (1932) published an illustrated account of some species from the Western Himalayas. Nevertheless, all those species stand in need of reinvestigation and careful revision. Besides, the excellent attempt by Schiffner (1910) to de-limit the generic boundaries of *Chiloscyphus* and the segregation of the new genus *Heteroscyphus* has not been taken into consideration either by Kashyap or Stephani; although the latter genus has been recognized by a number of hepaticologists in America and Japan and has also been included in a recent classification of hepatics by Evans (1939). Following Schiffner's concept (Schiffner 1910), *C. polyanthus* and *C. campanulatus* should be retained in the genus *Chiloscyphus* (Corda) Schffn. Two other species, *C. gollani* St. and *C. himalayensis* St., referred by Schiffner (1910) to this genus, are unfortunately unrepresented in our collections and it is not possible for the authors to discuss them. Two other species, viz. *C. flaccidus* and *C. gammianus*, also await detailed investigation and an attempt is being made to obtain the type specimens of these species from Stephani's herbarium for investigation. Another species, *C. inflatus*, instituted by Stephani (1906–1909) on the basis of specimens from the Himalayas shows entire leaves and amphigastria and has very conspicuous trigones in the leaf cells as well as in the cells of the amphigastria. From its overall appearance and details it is highly probable that this species may have to be removed from its present association with *Chiloscyphus* and *Heteroscyphus*. The remaining four species, viz. *C. argutus*, *C. coalitus*, *C. perfoliatus* and *C. communis*, have been referred by Schiffner (1910) to his genus *Heteroscyphus*.

The present communication has been possible from an investigation of numerous authentic specimens represented in 'PANDÉ COLLECTION', viz. an excellent collection of the East Himalayan Hepatics from the famous herbarium of E. Levier and determined by such eminent hepaticologists as Schiffner and Stephani, a collection from the Western Ghats by Pfleiderer of (Esslingen) Germany, a collection by Fr. Foreau from South India and some specimens from friends from different parts of the country along with a large number of specimens collected by Pandé himself. A key for the identification of the species of *Chiloscyphus* and *Heteroscyphus*, known from India, is given below:

Key for Identification

I. *Androecia intercalary*, on the main stem or on side branches; perigonial leaves saccate with a toothed dorsal flap and almost similar to the sterile leaves .. *Chiloscyphus*

A—Leaves entire

    (*a*) slightly imbricate or often not imbricate,
        semicircular to subquadrate ..   ..   (1) *C. polyanthus*

    (*b*) more or less closely imbricate, quadrate to
        oblong or oval

            + closely imbricate   ..   ..   (2) *C. himalayensis*

        + + slightly imbricate   ..   ..   (3) *C. campanulatus*

     + + + imbricate, broadly ovate, often
            apex obliquely retuse or un-
            equally obtusely bilobed   ..   (4) *C. gollani*

B—Leaves bidentate   ..   ..   ..   (5) *C. flaccidus**

C—Leaves pluridentate   ..   ..   ..   (6) *C. gammianus**

    II. *Androecia terminal*, on branches arising lateral-
ly from the angles of amphigastria; perigonial leaves
smaller and different from sterile leaves   ..   ..   *Heteroscyphus*

A—Leaves entire ..   ..   (1) *H. perfoliatus*

B—Leaves bidentate

    (*a*) cells at apex *c*. 27 μ, at base 36 $\mu \times$ 72 μ ..   (2) *H. communis*

    (*b*) cells at apex *c*. 36 μ, at base 54 $\mu \times$ 90 μ ..   (3) *H. coalitus*

C—Leaves pluridentate   ..   ..   ..   (4) *H. argutus*

DESCRIPTION

Chiloscyphus (Corda) Schiffner

Recently Schuster (1953) has ably discussed the generic features of
Chiloscyphus. According to him the genus is characterized by entire leaves,
with rhizoids restricted to small areas at the base of the under-leaves, ex-
tremely thin-walled leaf cells devoid of trigones, oil bodies composed of many
small, individually protruding oil globules, by the deeply three-lobed perianth
(with calyptra usually extending beyond it) on condensed lateral shoots and
male bract with its antical base having a small inflexed tooth or lobe.

1.   *Chiloscyphus polyanthus* (L.) Corda

Dioecious, caespitose forming brownish green patches on wet ground,
stem 3-4 cm long, often larger, prostrate, ascending or erect, sparingly
branched, branches irregular. *Rhizoids* hyaline, aggregated at the base of
the under-leaves; *leaves* 1 × 0·8 mm long, alternate, imbricate, longitudinally
inserted, slightly convex, *apex* entire and rounded; *cells at apex* 16·8 $\mu \times$ 25·2 μ
−21 $\mu \times$ 33·6 μ, *in the middle* 25 $\mu \times$ 16 μ, and *at base* 16·8 $\mu \times$ 29·4 μ−21 $\mu \times$ 37 μ,

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* Species tentatively recognized under *Chiloscyphus*.
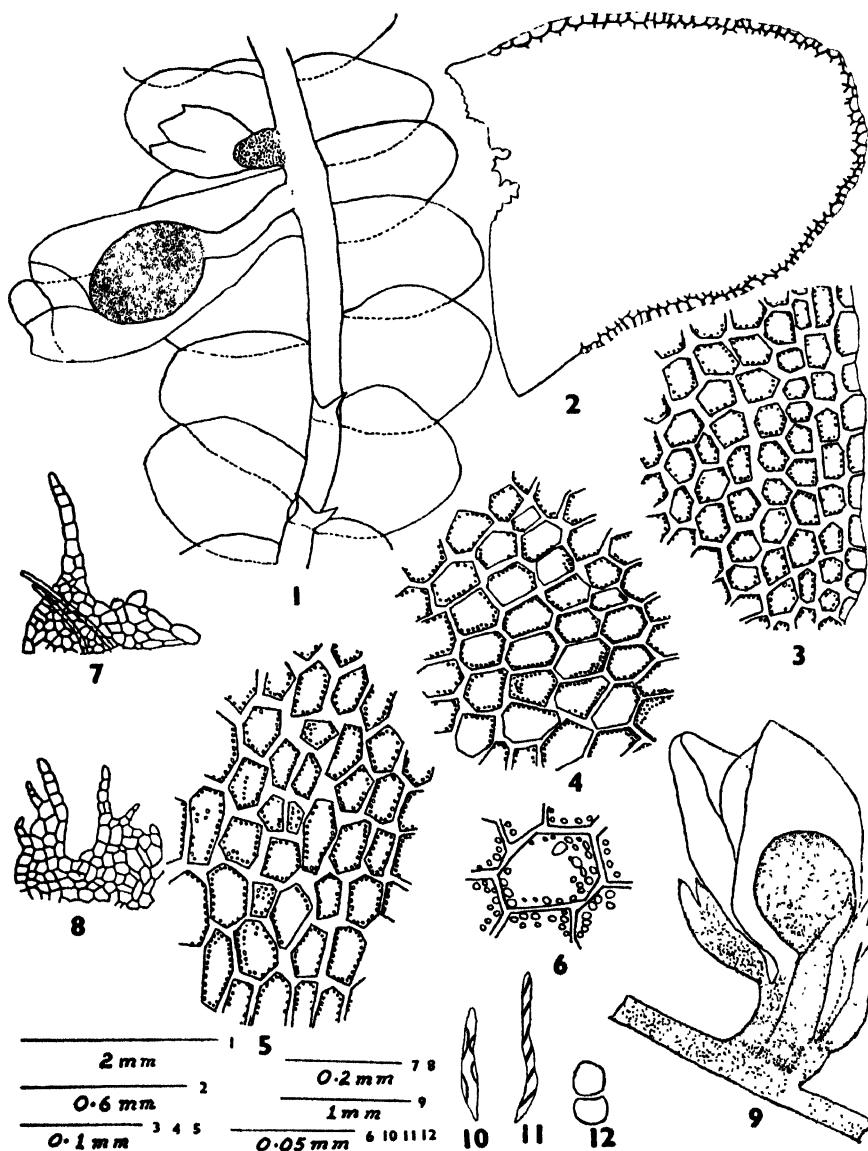
FIGS. 1–12. *Chiloscyphus polyanthus* (L.) Corda.

Fig. 1. Ventral view of the plant showing amphigastria and sporophytes. Fig. 2. Leaf with incurved margin. Figs. 3–5. Cells of the leaf from apex, middle and base respectively. Fig. 6. A cell. Note abundant oil bodies. Figs. 7-8. Amphigastria. Fig. 9. Female branch with mature capsule. Fig. 10. Elater (young). Fig. 11. Same (mature), monospiral. Fig. 12. Spores.

cell-walls thin, *trigones* absent. *Amphigastria*, small, distant, more or less deeply lobed, segments subulate, margin entire or with one or two teeth on either side of the lobes. *Female branches* numerous on very short laterals. *Involucral bracts* very much smaller than the leaves, bilobed, acute, three; bracteoles comparatively much smaller in size, three. *Bracts and bracteoles* closely appressed to the perianth. *Perianth* goblet-shaped, divided into three lobes. Lobule with entire margins. *Capsule* subtended on a long pedicel, oval, black at maturity. *Spores* 13·2 μ in maximum diameter, yellowish-brown or golden-yellow, tetrahedral. *Elaters* 88 μ, reddish-brown to yellow; mono- or bi-spiral or spirals totally absent, when absent the walls become thicker at intervals.

2. *C. campanulatus* St.

Dioecious, pale green, laxy-caespitose, intermingled with other liverworts and mosses. *Stem* about 2–4 cm long, sparingly branched. *Leaves* imbricate, divergent, alternate, ovate, entire, rounded, with entire margin, 1·1 mm long and 0·9 mm broad. *Cells at apex* 26·4–33 μ × 26·4–33 μ, *in middle* 39·6 μ × 30·7 μ and *at base* 33–42·9 μ × 30·7–34·3 μ. *Trigones* absent. *Amphigastria* small, bilobed, lobes narrow, sinus obtuse with one tooth on either side. *Perianth* formed by the fusion of three lobes on lateral branches, campanulate. *Involucral bracts* three, entire, notched, acute, reaching up to half the length of the perianth, *bracteoles* smaller, three, attached below the bracts, acute, notched. Bracts, bracteoles and perianth fuse to form a compact mass at the base of the sporophyte, through which seta emerges. *Capsule* oval or spherical, black at maturity, dehiscing by four valves, seta long and stout, occasionally included in the perianth. *Spores* golden-yellow, smooth; triradiate mark distinct, 13 μ in maximum diameter, elaters 145 μ long, bispiral, spirals black.

Heteroscyphus Schffn.

The genus *Heteroscyphus* is nearly identical to *Chiloscyphus* in its vegetative organization. The main differences, however, lie (i) in the position of the androecia (which are *terminal*) arising from the axils of amphigastria and (ii) in the perigonial leaves being smaller and different from sterile leaves.

3. *Heteroscyphus argutus* (Nees) Schffn.

Dioecious, terrietrial, whitish green in colour, mesophytic, growing on humid soil or on bark. Stem 2–5 cm long, sometimes up to 10 cm, simple or occasionally branched, erect or prostrate; *leaves* alternate, longitudinally. inserted, overlapping, 1·5–2 mm long, slightly imbricate, quadrate to rectangular or ovato-quadrate, *apex* toothed, teeth varying from 2–13 in number; *cells at apex* 22 μ × 19·8 μ, *in the middle* 26·4 μ × 26 μ and *at the base* 33·3 μ × 26·4 μ; *trigones* absent. *Oil bodies* numerous. *Amphigastria* cauline, small with
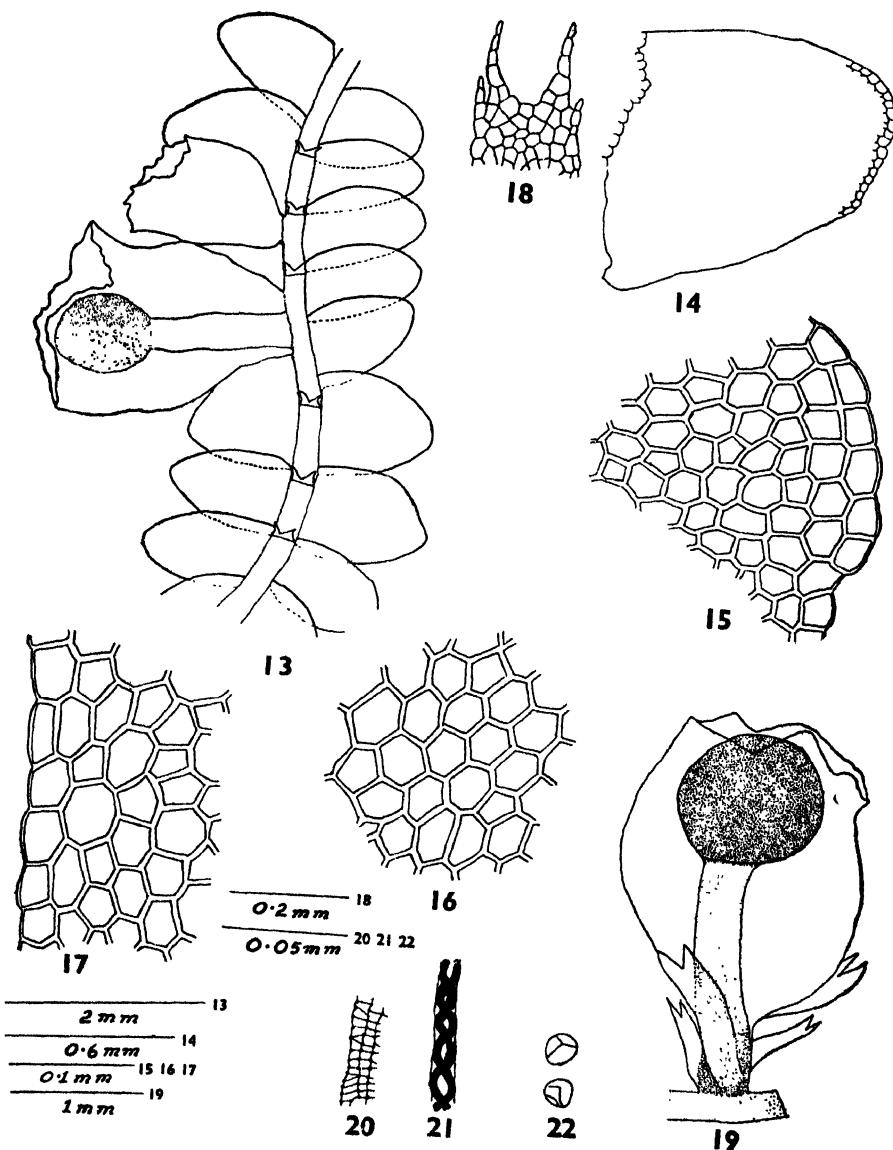
FIGS. 13–22.   *Chiloscyphus campanulatus* St.

Fig. 13.   Ventral view of the plant.   Fig. 14.   Leaf.   Figs. 15–17.   Cells of the leaf from apex, middle and base respectively.   Fig. 18.   Amphigastrium.   Fig. 19.   Campanulate perianth.   Fig. 20.   Capsule wall.   Fig. 21.   Elater, bispiral.   Fig. 22.   Spores.
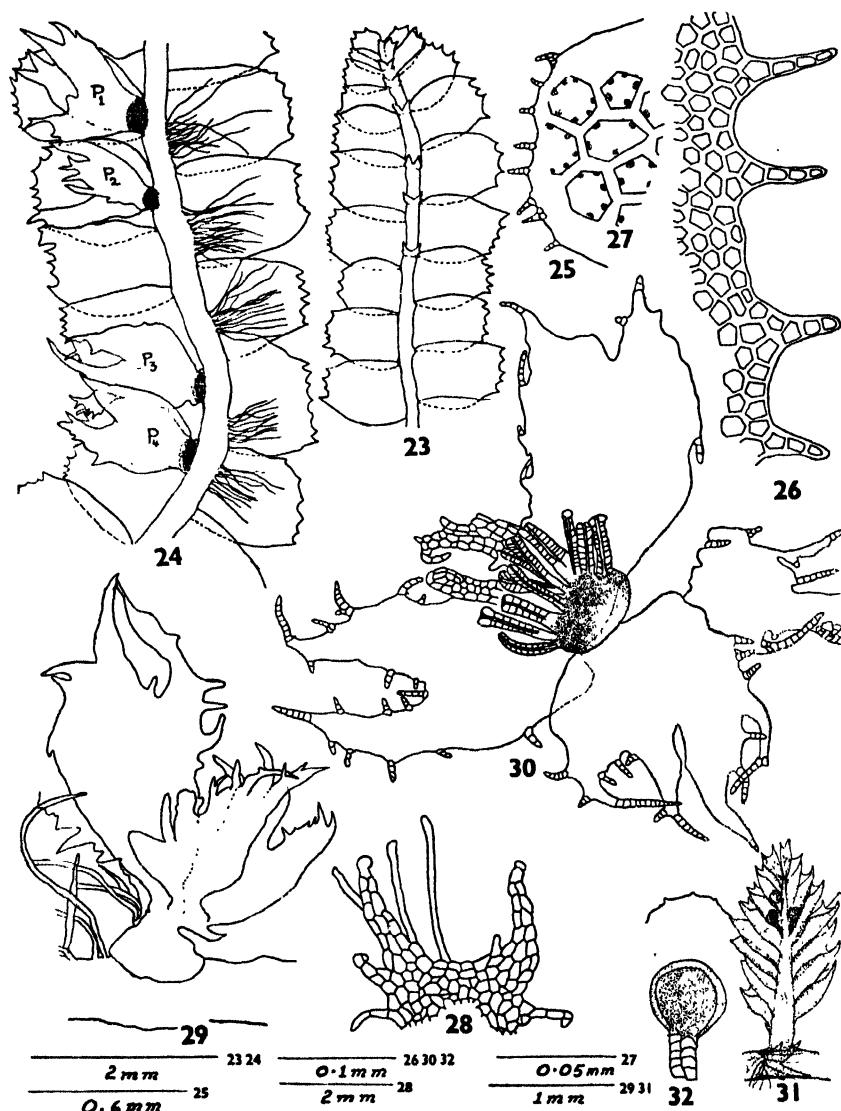
FIGS. 23–32.   *Heteroscyphus argutus* (Nees) Schffn.

Fig. 23.   Ventral view of the plant.   Fig. 24.   Same, showing female inflorescence.   Fig.
25.   Dentate leaf apex.   Fig. 26.   Same, magnified.   Fig. 27.   Cells with oil bodies.
Fig. 28.   Amphigastrium.   Fig. 29.   Female branch.   Fig. 30.   Perianth dissected
out.   Note numerous archegonia.   Fig. 31.   Male branch.   Antheridia (σ) on lateral
branch.   Fig. 32.   Antheridium.

broad base, oblong-lanceolate, deeply lobed and each lobe having one or two teeth on the margin. *Rhizoids* arising at the base of amphigastria, hyaline, aggregated. *Female inflorescence* on short laterals, *sporophyte* single or in groups of 2–4, hidden in leaves on either side of the stem. *Involucral bracts* as large as the leaves, shortly bilobed, lobes acute with many teeth, each tooth varying from 2–10 cells. *Bracts* three, long, bilobed, *bracteoles* small, as many as the bracts. *Archegonia* in clusters, 20–25 or more, normally only one attaining maturity, the rest degenerating. *Male branches* lateral, *bracts* smaller or of the same size as the vegetative leaves, usually 6–10 pairs, saccate, bidentate, each subtending an antheridium; antheridium grey to black at maturity, $99 \mu \times 46 \mu$, stalk 36–40 μ long.

H. argutus has an interesting distribution pattern and is confined to the Indo-Malayan region and Japan.

4. *H. coalitus* (Hook.) Schffn.

Dioecious, whitish green in colour, on wet soil or on bark intermingled with mosses and other liverworts. Plants large; *stem* usually 2 cm long, sometimes up to 5 cm or more. *Leaves* opposite, or subopposite, 1·3–2 mm long and 0·9–1 mm broad, dense, ascending, imbricate, margin entire, *apex* bidentate, *cells at apex* 19·8 $\mu \times 33 \mu$, *in middle* $33 \mu \times 26 \mu$ and *at base* $39 \mu \times 30 \mu$, *trigones* absent, cell walls thin. *Amphigastria* cauline, 0·54 mm long and 0·3 mm broad, deeply bilobed, one tooth on either side of the lobes. *Female plants* scarce, *perianth* large, ovato-campanulate, trilobate, lobes long, with more or less entire margins, often with few serrations at the apices; *bract and bracteole* each three in number. *Capsule* oval, subtended on a long seta, dehiscing into four valves. *Androecia* numerous, on main or lateral branches; *bracts* saccate, each subtending only one antheridium.

Schiffner (1910) treated two other of our species, *C. perfoliatus* and *C. communis* under the genus *Heteroscyphus*. Of these the latter is also known from Japan. Unfortunately specimens of these species are not represented in our collection.

OIL BODIES

Among the species of *Chiloscyphus* and *Heteroscyphus* described in the present communication, oil bodies occur in the following species:—

Chiloscyphus polyanthus.—Numerous oil bodies occur appressed to inner walls and all of these are approximately equal in size, averaging 6·6 μ across. In the Japanese specimens (Hattori 1951), however, these are larger being 8–15 $\mu \times 8$-9 μ.

Heteroscyphus argutus.—The oil bodies are fewer in number and occur as in *C. polyanthus*. These are, however, much smaller being 1·6–1·8 μ and

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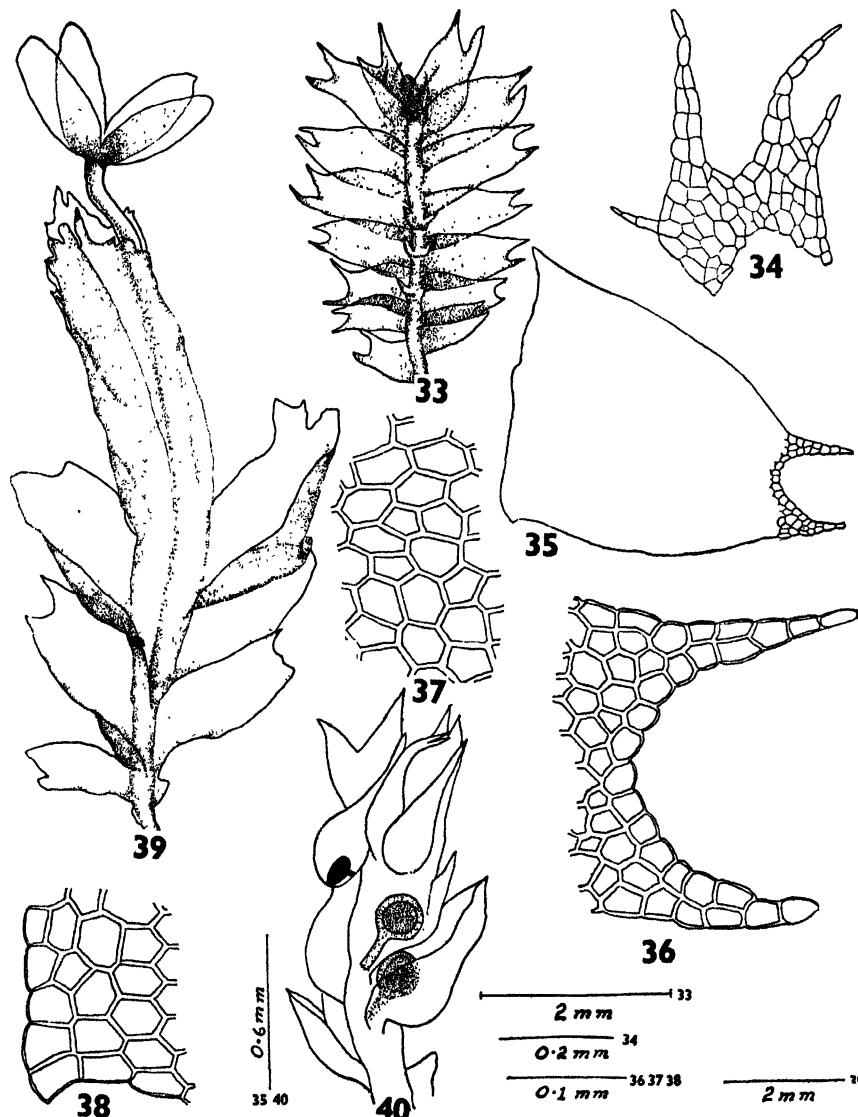
FIGS. 33–40.   *Heteroscyphus coalitus* (Hook.) Schffn.

Fig. 33.   Ventral view of the plant.   Fig. 34.   Amphigastrium.   Fig. 35.   Bidentate leaf.
Figs. 36–38.   Cells of the leaf from apex, middle and base respectively.   Fig. 39.
Female branch with dehisced capsule.   Fig. 40.   Male branch bearing antheridia.

are spherical or globular.   They occur not only in the cells of the leaves but occasionally also in the cells of the amphigastria.

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ADRENAL CORTICAL FUNCTION AND AMINOACIDURIA OF CARBON-TETRACHLORIDE-INDUCED CIRRHOSIS

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ABSTRACT

The role of adrenal cortex in the aminoaciduria of carbon-tetrachloride-induced cirrhosis was studied in albino rats. Adrenal cortical function and plasma and urinary amino-acids were studied during the progressive evolution of the cirrhosis and the results were correlated. The data obtained have demonstrated the presence of a close association between abnormalities in plasma and urinary amino-acids and adrenal cortical dysfunction in this disease.

INTRODUCTION

Aminoaciduria is a frequent feature in experimental (Himsworth and Glynn 1945; Dent 1947) and in human liver disease (Dent 1947; Gabuzda *et al.* 1952; Walshe 1953; Ramachandran *et al.* 1960). Defective deamination of the amino-acids as well as their impaired incorporation into proteins (Kinsell *et al.* 1948) may be the principal factors causing their increased levels in blood (Kirsner *et al.* 1950; Walshe 1953) which then results in their increased urinary excretion. However, aminoaciduria is not consistently seen in every case of liver disease (Kirsner *et al.* 1950) and this suggests that other factors too may be involved in this phenomenon.

It has been demonstrated recently that adrenal cortical functional insufficiency exists in experimental (Wahi *et al.* 1956, 1960a) and in human liver disease (Brown *et al.* 1954; Wahi *et al.* 1957, 1960b; Wahi and Ramachandran 1958). As there is evidence that adrenal cortical hormones promote deamination of amino-acids (Samuels *et al.* 1936; Russell and Wilhelmi 1941) as well as their incorporation into plasma and tissue proteins (Russell 1955), it is possible that the existing state of adrenal cortical hypofunction also contributes towards the production of aminoaciduria of liver disease. In an attempt to test this hypothesis the present experimental study in albino rats was carried out.

MATERIALS AND METHODS

A total of 200 albino rats in the weight range 80–120 gm. were injected subcutaneously twice weekly with 0·1 c.c. of carbon tetrachloride in an equal volume of liquid paraffin. The animals were continued on the stock diet during the experimental period.

A batch of 4-5 rats was isolated at the end of every week of the experiment.   24-hour urine specimens were then collected and volume measured.

The rats were anesthetized with ether and blood was drawn out by cardiac puncture.   The liver and adrenal glands were taken out.

Histological studies of the liver tissue were done using Hematoxylin and Eosin, Masson's and Reticulum stains, and that of adrenal gland by using Hematoxylin and Eosin and Sudan IV stains.

The serum was deproteinized with 4 volumes of 80 per cent alcohol (Bernstein *et al.* 1956) centrifuged and the centrifugate restored to original volume by evaporating off the alcohol.   0·05 c.c. of this protein-free serum was applied to Whatman No. 1 filter circle diameter 38 cm.   5 microlitres of hydrogen peroxide (30 per cent) were also added to the spot to oxidize the cystine to cysteic acid, and the methionine, to methionine sulphoxide. Chromatography was then carried out using butyl alcohol-acetic acid-water (4 : 1 : 5) as the solvent (Wahi *et al.* 1954).   After spraying the developed chromatogram with 0·25 per cent ninhydrin in butanol, the amino-acid spots were cut out from the paper, eluted with 75 per cent ethanol (containing 0·05 mg. $CuSo_4.5H_2O$ per c.c.) and the colours measured in a Klett-Summerson photoelectric colorimeter.   From the colorimetric readings the values were read off a standard curve and concentrations of individual amino-acids in mg. per 100 c.c. serum calculated.

Similarly, 0·025 c.c. of the 24-hour urine specimen was chromatographed, without initial deproteinization, and the individual amino-acid excretion levels for the 24-hour period calculated from the colours of the amino-acid-ninhydrin spots, as described for serum.

Simultaneously, during each week of the experimental period adrenal cortical functions were assessed by the adrenal ascorbic acid depletability technique, using ACTH (1 mg. per 100 gm. body weight) as the stress (Wahi *et al.* 1956).

OBSERVATIONS AND RESULTS

Evidence of fibrosis in the liver was first seen in the 4th week of the experimental period which gradually increased during the progressive aminoaciduria.   This fibrosis was graded into five groups:

1 plus Fibrosis: The general architecture of the liver was intact and the vascular landmarks in their normal positions.   Many lobules showed fine streaks of condensed reticulum originating from the central veins and running for short distance radially across the liver lobule.   Portal tracts rarely showed any condensation of reticulum.   With Hematoxylin and Eosin stain the liver cells showed moderate to marked degree of parenchymal damage and focal areas of necrosis, chiefly in the central zone of the liver lobule.

2 plus Fibrosis: The lobular pattern was still intact and the vascular

landmarks normally placed anatomically. The condensation of reticulum in radial streaks from the central vein was relatively more marked; only rarely small prolongations of thickened reticulum from the portal tracts running in irregular directions joined these bands.

3 plus Fibrosis: The earliest tendency to pseudolobulation was seen. Slender prolongations of thickened reticulum from the portal tracts were seen here more frequently. At places these joined bands originating around the central veins and enclosed irregular groups of liver cells, in which the normal relationship of the portal tracts and central veins was lost.

4 plus Fibrosis: The normal lobular architecture of the liver was completely disarrayed by bands of varying thickness of fibrous tissue traversing the liver parenchyma irregularly. The pseudolobules varied much in size and shape. These fibrous tissue bands showed marked evidence of neoangiogenesis and diffuse infiltration by a moderate number of mononuclear cells.

5 plus Fibrosis: This represented an exaggeration of changes seen in grade 4 plus. The pseudolobules were smaller in size, with the fibrous tissue bands much thicker. These showed evidence of hyaline change in several places and continued to be vascular although the degree of cellularity diminished appreciably. Portal tracts and central veins were not identified with certainty as they were incorporated in the fibrous tissue bands.

The results of the serum and urinary amino-acid studies during each week of the experimental period are presented in Tables I and II and Charts I and II. The data obtained in the adrenal function studies are given in Table III and Charts I and II.

In the paper chromatograms of normal serum and urinary specimens the following amino-acid spots could be detected: Cystine, glutamine, glycine-serine-aspartic acid, glutamic acid, alanine, tyrosine, valine and leucine. In addition methionine could often be detected in chromatograms of normal urines. Practically the same number of amino-acids were visible on the chromatograms of blood specimens of rats sacrificed at weekly intervals up to the end of the experimental period. The only significant observation was that methionine was visible on the chromatograms only from 10th week of experimental period.

Quantitatively, however, there were considerable differences in the effects of advancing cirrhosis on the serum and urinary levels of various amino-acids (Tables I and II; Charts I and II).

There was considerable impairment of adrenal cortical function from 10th week onwards (Table III; Charts I and II).

Serum and urinary cystine levels showed no significant differences from normal till the end of 9th week. From 10th week onwards there was a large increase in the levels of this amino-acid (Tables I and II; Charts I and II).

Chart I:— RELATIONSHIP OF SERUM AMINOACID CHANGES TO ADRENAL CORTICAL FUNCTION DURING EVOLUTION OF CARBON TETRACHLORIDE CIRRHOSIS IN ALBINO RATS.
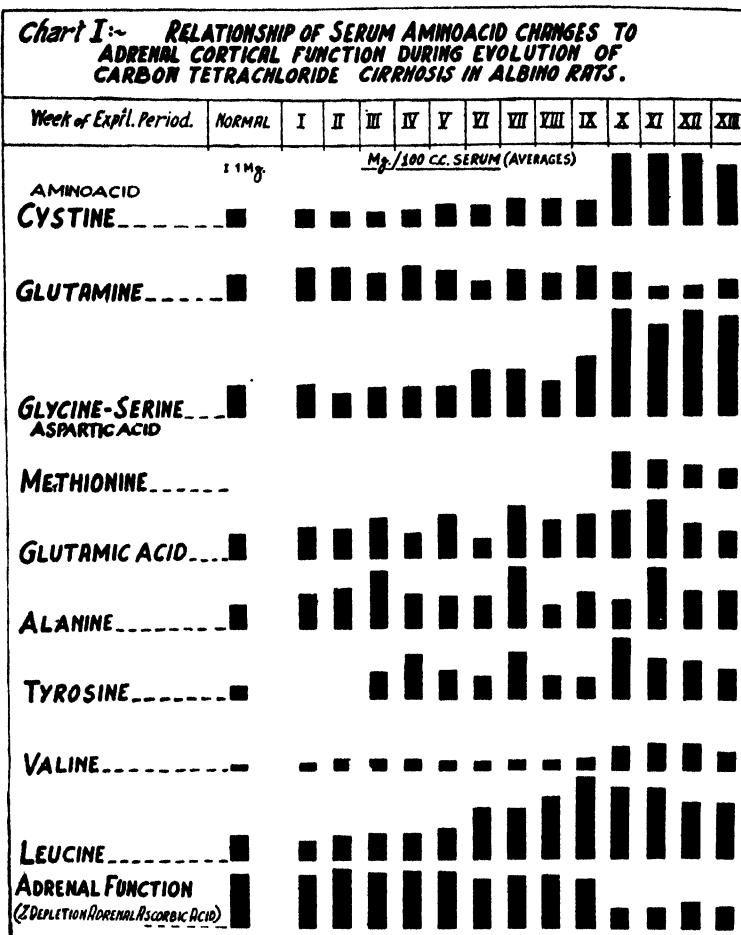
Serum and urinary glutamine levels showed no large differences throughout the experimental period.

Glycine-Serine-Aspartic acid levels in serum and urine showed variable increases up to the end of 9th week. There was a sudden large increase in the levels of these amino-acids with the onset of adrenal cortical insufficiency from the 10th week (Tables I and II; Charts I and II).

Serum and urinary methionine levels were also elevated from 10th week of the experimental period.

Glutamic acid levels in serum and urine showed no consistent increases during the various stages of the experiment and there was no relationship between these and adrenal cortical responsiveness (Tables I and II; Charts I and II).

Serum and urinary levels of alanine were elevated during most weeks of

Chart II :— RELATIONSHIP OF URINARY AMINOACID CHANGES TO ADRENAL CORTICAL FUNCTION DURING EVOLUTION OF CARBON TETRACHLORIDE CIRRHOSIS in ALBINO RATS.
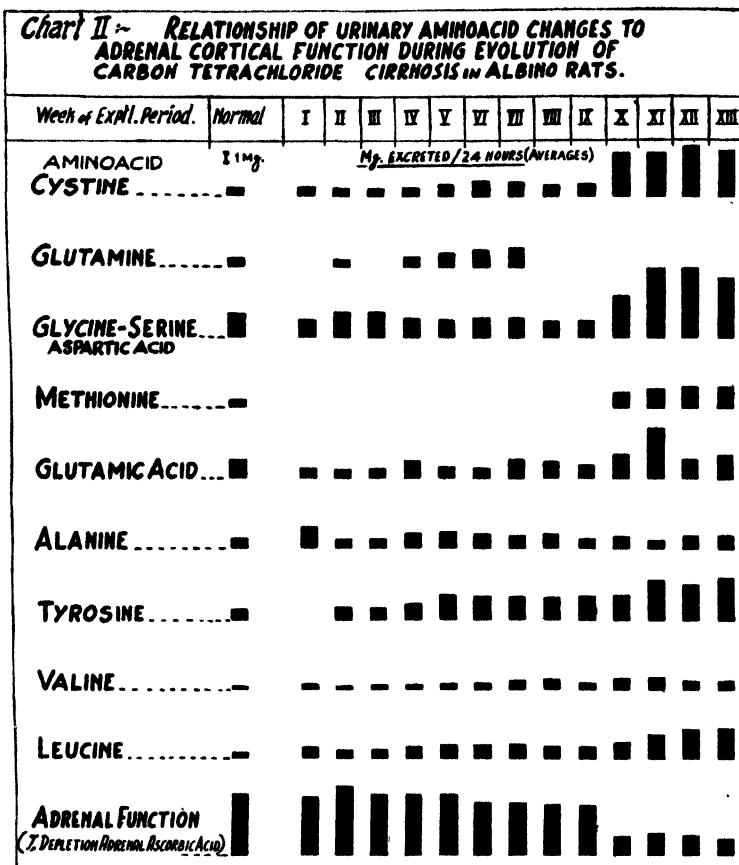
the experimental period though these increases were not consistently seen (Tables I and II; Charts I and II).

Tyrosine levels in serum and urine tended to be elevated from the beginning of the experimental period. There were further increases in the blood and especially urinary tyrosine levels with onset of adrenal cortical insufficiency from 10th week (Tables I and II; Charts I and II).

Serum and urinary levels of valine showed variable increases from the beginning of the experimental period. From 10th week onwards further elevation in blood and urinary values of this amino-acid was seen (Tables I and II; Charts I and II).

Serum leucine levels were increased from about the 6th week of the experimental period. There was no marked effect of deterioration of adrenal function from 10th week onwards on the serum level of this amino-acid. Urinary leucine levels were increased from beginning of the experimental period and there was again no evidence of a relationship between these and adrenal function (Tables I, II and III; Charts I and II).

TABLE I

Serum amino-acid changes during evolution of carbon tetrachloride cirrhosis in albino rats

Amino-acid	Normal values (averages) Mg. per 100 c.c. serum	Week of experimental period — Mg. per 100 c.c. serum (averages)												
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Cystine	3·5	3·0	3·8	3·1	3·2	4·4	4·0	5·2	4·5	4·20	9·30	9·0	9·2	7·0
Glutamine	4·0	6·0	6·0	4·5	6·0	5·2	3·0	5·5	4·5	6·0	4·5	1·5	1·8	3·1
Glycine-Serine-Aspartic acid	7·7	8·0	4·5	6·5	6·1	8·9	12·0	14·4	10·0	16·0	27·0	20·0	25·0	21·0
Methionine	··	··	··	··	··	··	··	··	··	··	6·3	5·0	4·8	3·2
Glutamic acid	4·1	5·2	4·4	7·5	4·4	7·8	3·5	9·5	6·8	7·5	8·8	9·5	6·8	5·0
Alanine	3·6	5·0	7·2	10·0	5·2	4·9	5·0	11·0	3·2	6·0	4·7	9·1	6·0	6·1
Tyrosine	2·2	··	··	4·0	7·2	5·2	4·0	8·0	4·4	4·0	12·0	8·2	7·8	6·1
Valine	0·7	0·8	1·2	1·2	1·2	0·98	1·0	1·1	0·8	1·2	3·3	3·7	3·8	2·5
Leucine	4·0	2·2	3·3	4·0	3·8	4·8	7·2	7·2	8·2	10·0	9·0	9·0	7·2	7·6

TABLE II

Urinary amino-acid changes during evolution of carbon tetrachloride cirrhosis in albino rats

Amino-acid	Normal values (averages) Mg. per 24 hrs.	Week of experimental period — Mg. per 24 hours (averages)												
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Cystine	0·30	0·30	0·26	0·26	0·26	0·48	0·88	0·50	0·40	0·52	2·45	2·5	2·81	2·64
Glutamine	0·45	..	0·70	..	0·65	0·90	1·35	1·30	..	..	..	..	..	..
Glycine-Serine-Aspartic acid	3·10	1·50	4·00	3·75	5·20	3·30	4·06	3·00	1·60	2·86	10·5	20·0	20·0	13·2
Methionine	0·54	..	..	..	..	..	..	..	..	..	1·55	2·24	2·24	2·20
Glutamic acid	1·70	0·90	0·64	0·60	1·17	0·90	0·91	1·50	1·00	1·04	2·10	4·00	1·60	1·76
Alanine	0·55	2·10	0·64	0·60	1·17	2·40	1·35	1·35	1·00	1·20	0·87	0·57	1·30	1·32
Tyrosine	0·75	..	0·64	0·70	1·30	2·40	2·26	2·10	2·06	2·60	2·81	4·00	3·20	4·44
Valine	0·13	0·30	0·16	0·15	0·18	0·18	0·14	0·30	0·40	0·26	1·40	2·00	0·60	1·10
Leucine	0·37	0·90	0·64	0·60	0·91	0·90	0·78	1·40	0·93	0·83	1·26	1·67	2·00	2·26

TABLE III

Adrenal cortical function during evolution of carbon tetrachloride cirrhosis in albino rats

Week of experimental period	Depletability of adrenal ascorbic acid (average %)
Normal	59
1	55
2	58
3	52
4	52
5	49
6	45
7	48
8	47
9	42
10	15
11	13
12	18
13	17

DISCUSSION

The results obtained in the present study indicate that the functional status of the adrenal cortex probably is related to the aminoaciduria of liver disease. It is apparent that the blood and urinary levels of certain amino-acids show further sudden elevations simultaneously with the functional impairment of the adrenal cortex from the 10th week of experimental period. Particularly the key amino-acids, cystine and methionine, are apparently influenced by deficiency of the adrenal cortical hormones and this is of considerable clinical significance. This suggests that the diminished utilization of at least some of these amino-acids in liver disease, as shown by their elevated blood and urinary levels, may at least partly be related to the state of adrenal hypofunction in this diseased state.

In liver disease, both the blood and urinary levels of amino-acids are elevated (Dent and Walshe 1954). Hence the aminoaciduria is probably not due to renal damage but a consequence of raised blood levels.

The blood levels of amino-acids may be increased due to one or more of the following factors: (i) increased release from tissue proteins, (ii) impaired deamination in liver, (iii) decreased incorporation into blood and tissue proteins and (iv) diminished conversion to urea and other non-protein nitrogen compounds. Since all these processes take place primarily, if not solely, in the liver, functional impairment of the liver can cause all these abnormalities. Hence in the initial stages of the disease, at least, the aminoacidemia and aminoaciduria are due to liver functional damage.

There is evidence in the literature to show that adrenal cortical hormones too influence several of these factors. Russell and Wilhelmi (1941) using kidney slices from adrenalectomized rats have shown that glycogen formation from administered DL-Alanine was less than normal. This would imply that in the absence of these hormones deamination of amino-acids is impaired. It is of interest in this connection that cortisone administration has been shown to cause elevated tryptophane peroxidase activity in the liver of intact rats (Mehler et al. 1958) and also to restore to normal the diminished arginase activity in liver of adrenalectomized rats (Bach et al. 1958).

Cortisone administration promotes incorporation of glycine into liver proteins (Lebedeva 1956). Similarly, the adrenal cortex is apparently necessary for incorporation of cystine into liver proteins (Aschkenasy 1957). Clark (1953) has reported that incorporation of C14-labelled amino-acids into the proteins of the liver and of the plasma was increased considerably in rats treated with cortisone. These studies suggest that in conditions of adrenal cortical insufficiency, such as exist in liver disease, there will be impaired amino-acid incorporation into liver proteins.

Several observations indicate that when adrenal cortical activity is increased the excretion of labelled amino-nitrogen as urea is also markedly elevated (Hoberman 1950; Parson et al. 1952; Clark 1953). This would suggest that the conversion of amino-nitrogen to urea is under adrenal cortical influence. Likewise incorporation of administered cystine into liver glutathione has been shown to be inhibited in adrenalectomized rats (Wellers and Aschkenasy 1957).

It is evident from the above-mentioned studies that, at least in the case of some amino-acids, adrenal cortical hormones promote their incorporation into urea, peptides, and proteins as well as their degradation in the body. Hence adrenal cortical insufficiency can be expected to cause elevated levels of these amino-acids. This is probably the mechanism underlying the relationship between blood and urinary amino-acid changes and adrenal cortical function in liver disease, as observed in the present study. If the presence of adrenal cortical hypofunction is the cause of the aggravation of amino-aciduria from 10th week onwards, then theoretically at least administration of cortisone or allied hormones would be expected to reverse the above effect. But since it is known that prolonged cortisone administration causes further depression of adrenal function (Selye 1947), this aspect of the study has not been considered practicable.

While the initial defect causing the elevated blood and urinary amino-acid changes appears to be liver cell failure, at a later stage the effect of adrenal cortical insufficiency is probably superimposed on this leading to more marked amino-acid abnormalities.

SUMMARY

The relationship of functional abnormalities in the adrenal cortex to the amino-acid changes in carbon-tetrachloride-induced cirrhosis was assessed by studying the serum and urinary levels of amino-acids during the evolution of the cirrhosis in albino rats, using the technique of paper partition chromatography, and correlating these with the state of adrenal cortical function.

The data obtained in this way showed that onset of adrenal cortical insufficiency was accompanied by further aggravation of the already raised blood and urinary amino-acid levels.

The results of the present study suggest therefore the presence of an association between adrenal cortical dysfunction and aminoaciduria of carbon-tetrachloride-induced cirrhosis. The question whether there is a cause-and-effect relationship between these two phenomena requires to be elucidated.

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OBSERVATIONS ON AUTOHAEMOLYSIS IN THALASSAEMIA SYNDROME

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ABSTRACT

When blood from normal subjects was incubated at 37° C. under sterile conditions, little or no haemolysis took place within 48 hours. Under similar conditions, blood from patients with Hb. E-thalassaemia disease and homozygous thalassaemia showed an increased rate of haemolysis. Slightly increased rate of haemolysis was also seen in thalassaemia trait. Autohaemolysis was not associated with any change in the pH of the blood.

INTRODUCTION

It has been observed that, when normal human blood is incubated at 37° C. under sterile conditions, little or no haemolysis takes place within 48 hours (Dacie 1960). Studies on haemolytic anaemias have shown that, in hereditary spherocytosis, the rate of *in vitro* haemolysis is markedly increased (Dacie 1941, 1943) and that this haemolysis is more marked in those cases which show high osmotic fragility of unincubated blood. Similar results have been reported in acquired haemolytic anaemia (Dacie 1950; Young *et al.* 1956), paroxysmal nocturnal haemoglobinuria (Dacie *et al.* 1938), in some proliferative disorders (Crosby and Benjamin 1957), hereditary elliptocytosis and some cases of congenital non-spherocytic haemolytic anaemia (Selwyn and Dacie 1954). Perusal of the literature shows that, in thalassaemia syndrome, the data pertaining to autohaemolysis are scanty, the only information available is that due to Selwyn (1960) who in one case of thalassaemia studied recorded values in the upper limit of normal.

The present communication is an account of studies on autohaemolysis in thalassaemia syndrome. The changes in the packed cell volume were noted in all cases and the pH of the blood as observed during the process was noted in some of these cases.

MATERIAL AND METHODS

Subjects.—The following were investigated: Hb. E-thalassaemia (46), homozygous thalassaemia (8), Hb. E-trait (18) and thalassaemia trait (29). In addition, 7 patients with iron deficiency and 18 normal subjects were studied as controls.

Methods.—Twenty ml. of venous blood were collected with necessary sterile precautions and defibrinated using 5 glass beads and shaking gently

in a sterile Erlenmeyer flask in order to avoid undue injury to the red cells. The blood was then transferred to four sterile flasks and incubated at 37° C. The contents of two of these flasks were studied after 24 hours and the remaining two after 48 hours following the method of Dacie (1950). Quantification of autohaemolysis was done as follows:

The incubated blood was centrifuged at a rate of 3,000 r.p.m. for 30 minutes in order to determine the packed cell volume. The supernatant serum of unincubated blood (R') and from incubated blood (R_1) was diluted (1 in 25 or 1 in 50) while the whole blood (R_0) was diluted (to 1 : 100 or in 200) using $\frac{N}{10}$ HCl as the diluent. The percentage of haemolysis was calculated using the formula

$$(R_T - R') \times \frac{\dfrac{100 - PCV_T}{100}}{(R_0 - R') \times 4} \times 100$$

where R_0 = optical density of diluted whole blood.

       R_T = optical density of diluted serum at time T (24 or 48 hours).

       R' = optical density of diluted unincubated serum.

PCV_T = packed cell volume at time T (24 or 48 hours).

A mean of 2 samples studied simultaneously was recorded.

Packed cell volume of the unincubated defibrinated blood was recorded by centrifuging the blood at 3,000 r.p.m. for 30 minutes. The values for 24 and 48 hours' incubation were obtained from the corresponding samples studied for autohaemolysis.

pH of blood.—The pH of whole blood was recorded immediately after withdrawal from the vein using Photovolt pH meter. The defibrinated blood and samples of blood incubated for 24 and 48 hours were similarly studied.

RESULTS

1. *Hb. E-thalassaemia*

     (a) *After 24 hours' incubation.*—Mean value for autohaemolysis was 1·98 per cent and the range varied from 0·3 to 4·0 per cent. In 36 cases, values above 1 per cent were recorded.

     (b) *After 48 hours' incubation.*—Mean value for autohaemolysis was 6·33 per cent and the range varied from 0·9 to 42·4 per cent. In 15 cases, values above 5 per cent were recorded.

Packed cell volume.—Mean value for PCV of defibrinated blood before incubation was 22 per cent and the range varied from 9 to 32 per cent. After 24 hours' incubation, the mean value was 23 per cent and the range varied from 9 to 40 per cent while after 48 hours' incubation the mean value remained 23 per cent and the range varied from 11 to 38 per cent.

pH of blood.—Mean value for the pH of fresh whole blood was 7·41 and the range varied from 7·30 to 7·60.   The mean value for defibrinated blood was 7·69 and the range varied from 7·50 to 8·00.   After 24 hours' incubation, mean value was 7·06 and range varied from 6·90 to 7·20 while after 48 hours' incubation, mean value was 7·03 and range varied from 6·90 to 7·50.

2.   *Homozygous thalassaemia*

Autohaemolysis after 24 hours' incubation.—Mean value was 3·08 per cent and the range varied from 0·8 to 6·9 per cent.   Values above 1 per cent were recorded in 6 patients.

Autohaemolysis after 48 hours' incubation.—Mean value was 6·19 per cent and the range varied from 1·6 to 12·5 per cent.   Values above 5 per cent were recorded in 5 patients.

Packed cell volume.—Mean value for *PCV* of defibrinated blood before incubation was 22 per cent and the range varied from 9 to 30 per cent.   After 24 hours' incubation, the mean value was 25 per cent and the range varied from 10 to 40 per cent while after 48 hours, the mean value was 28 per cent and the range varied from 14 to 45 per cent.

pH of blood.—Mean value for pH of whole fresh blood was 7·50 and the range varied from 7·3 to 7·7.   Mean value for defibrinated blood was 7·7 and the range varied from 7·6 to 8·1.   After incubation for 24 hours, the mean value was 7·26 and the range varied from 7·1 to 7·4 while after 48 hours, the mean value was 7·0 and the range varied from 6·9 to 7·1.

3.   *Hb. E-trait*

Autohaemolysis after 24 hours' incubation.—Mean value was 0·7 per cent and the range varied from 0·22 to 2·04 per cent.   In 5 cases values above 1 per cent were recorded.

Autohaemolysis after 48 hours' incubation.—Mean value was 2·33 per cent and the range varied from 0·84 to 6·5 per cent.   In 2 cases values above 5 per cent were obtained.

Packed cell volume.—Mean value for *PCV* of defibrinated blood before incubation was 42 per cent and the range varied from 31 to 46 per cent. After 24 hours' incubation, the mean value was 47·7 per cent and the range varied from 30 to 61 per cent while after 48 hours' incubation, the mean value was 47·9 per cent and the range varied from 30 to 60 per cent.

pH of blood.—Mean value for the whole fresh blood was 7·45 and the range varied from 7·30 to 7·70.   Mean value for defibrinated blood was 7·70 and the range varied from 7·60 to 7·85.   After 24 hours' incubation the mean value was 7·23 and range 6·8 to 7·7, while after 48 hours' incubation mean value was 7·18 and the range varied from 6·9 to 7·3.

4. *Thalassaemia trait*

Autohaemolysis after 24 hours' incubation.—Mean value was 0·89 per cent and the range varied from 0·08 to 2·9 per cent. In 10 cases values above 1 per cent were recorded.

Autohaemolysis after 48 hours' incubation.—Mean value was 3·68 per cent and the range varied from 0·40 to 18·7 per cent. In 4 of these cases values above 5 per cent were recorded.

Packed cell volume.—Mean value for the *PCV* of defibrinated blood before incubation was 37 per cent and the range varied from 30 to 46 per cent. After 24 hours' incubation, the mean value was 43·4 per cent and the range varied from 27 to 60 per cent while after 48 hours, the mean value was 46 per cent and the range varied from 27 to 65 per cent.

p*H of blood.*—Mean value for the whole fresh blood was 7·50 and the range varied from 7·4 to 7·7. Mean value for the defibrinated blood was 7·72 and the range varied from 7·6 to 7·9. After 24 hours' incubation the mean value was 7·15 and range from 6·75 to 7·50 while after 48 hours, the mean value was 6·97 and the range varied from 6·7 to 7·3.

5. *Iron deficiency anaemia*

Autohaemolysis after 24 hours' incubation.—Mean value was 0·29 per cent and the range varied from 0·12 to 0·6 per cent.

Autohaemolysis after 48 hours' incubation.—Mean value was 2·31 per cent and the range varied from 0·2 to 5·9 per cent. Value above 5 per cent was recorded in 1 subject only.

Packed cell volume.—Mean value for *PCV* of defibrinated blood before incubation was 20 per cent and the range varied from 15 to 31 per cent. After 24 hours' incubation, the mean value was 26 per cent and the range varied from 18 to 35 per cent while after 48 hours' incubation mean value was 21 per cent and the range was 15 to 32 per cent.

p*H of blood.*—The mean value for whole fresh blood was 7·46 and the range varied from 7·3 to 7·6. Mean value for defibrinated blood was 7·78 and the range varied from 7·6 to 8·0. After 24 hours' incubation, the mean value was 7·22 and range 7·0 to 7·4 while after 48 hours, mean value was 6·98 and the range varied from 6·8 to 7·1.

6. *Normal subjects*

Autohaemolysis after 24 hours.—Mean value was 0·5 per cent and range varied from 0·07 to 1·0 per cent.

Autohaemolysis after 48 hours.—Mean value was 1·53 per cent and the range varied from 0·51 to 3·8 per cent.

Packed cell volume.—Mean value for *PCV* of defibrinated blood before incubation was 47·5 per cent and the range varied from 41 to 50 per cent.

II

After 24 hours' incubation the mean value was 57 per cent and range from 46 to 70 per cent while after 48 hours' incubation the mean value was 47·6 per cent and the range varied from 38 to 56 per cent.

p*H of blood.*—Mean value for the whole fresh blood was 7·39 per cent and the range varied from 7·25 to 7·6. Mean value for defibrinated blood was 7·71 and the range varied from 7·55 to 7·90. After 24 hours' incubation, the mean value was 7·15 and the range varied from 6·9 to 7·2 while after 48 hours' incubation, the mean value was 7·07 and the range varied from 6·9 to 7·5.

Tables 1 and 2 show the results in brief and their statistical analysis.

TABLE 1

Per cent autohaemolysis after 24 and 48 hours' incubation

Group	24 hours' incubation			48 hours' incubation		
	No. of observations	Range	Mean	No. of observations	Range	Mean
Normal	18	0·09–1·00	0·50	17	0·51– 3·80	1·53
Hb. E-thalassaemia ..	46	0·30–4·00	1·98	41	0·90–42·4	6·33
Homozygous thalassaemia ..	8	0·8 –6·9	3·08	8	1·6 –12·5	6·19
Hb. E-trait ..	18	0·22–2·04	0·70	17	0·84– 6·50	2·33
Thalassaemia trait ..	29	0·08–2·90	0·89	29	0·40–18·70	3·68
Iron deficiency anaemia	7	0·12–0·60	0·28	7	0·20 –5·9	1·5

TABLE 2

Mean and standard errors of pH of blood

Subjects	Fresh whole blood	Defibrinated blood	After 24 hours' incubation	After 48 hours' incubation
Normal (11)	7·39 ± 0·0762	7·71 ± 0·042	7·15 ± 0·0695	7·07 ± 0·0377
Hb. E-thalassaemia (10) ..	7·41 ± 0·0333	7·69 ± 0·0473	7·06 ± 0·0364	7·03 ± 0·1536
Homozygous thalassaemia (3)	7·50 ± 0·1410	7·70 ± 0·4840	7·26 ± 0·2740	7·00 ± 0·0707
Hb. E-trait	7·45 ± 0·0683	7·70 ± 0·0447	7·23 ± 0·1570	7·13 ± 0·3955
Thalassaemia trait (4) ..	7·50 ± 0·0355	7·72 ± 0·1321	7·15 ± 0·0531	6·97 ± 0·1658
Iron deficiency anaemia (5)..	7·46 ± 0·2310	7·78 ± 0·0660	7·22 ± 0·800	6·98 ± 0·1830

DISCUSSION

From the above studies, it is clear that in *Hb. E-thalassaemia* the rate of autohaemolysis is high. The difference from the normal group was statistically significant giving a *t* value of 7·87 and $P < 1$ per cent for 24 hours' incubation and $t = 3·47$ and $P < 1$ per cent for 48 hours. But unlike the normal group, the degree of haemolysis at 48 hours could not be inferred

from the data obtained for 24 hours' incubation. It was found that in this group some cases showed low values for autohaemolysis during first 24 hours and in these cases values remained low even during the subsequent 24 hours while in others it was markedly accelerated. Some of those patients in whom the rate of autohaemolysis was faster during first 24 hours, maintained similar rate during the next 24 hours while in others it was further accelerated. When compared to iron deficiency anaemia significant differences were found. For 24 hours' incubation a t value of 3·74 and P value of 1 per cent was obtained. After 48 hours' incubation t value of 3·29 and P value of 1 per cent were obtained.

In homozygous thalassaemia the results were similar to those of Hb. E-thalassaemia. When compared to normal subjects, the t values of 3·65 significant at 5 per cent level and 3·70 significant at 5 per cent level were obtained after 24 and 48 hours' incubation respectively. When compared to iron deficiency anaemia, t values of 3·96 significant at 1 per cent level and 3·24 denoting significance at 5 per cent were obtained.

Hb. E-trait after 24 hours' incubation did not show any value significantly higher than normal. After 48 hours' incubation the mean value obtained was slightly higher than that of normals. On statistical analysis, this difference was not, however, found to be significant, the observed t value being 1·72 while significant t at 5 per cent level would be 2·04.

Thalassaemia trait.—The rates of autohaemolysis both after 24 and 48 hours were significantly higher than those obtained for normal subjects; the t values were 2·51 ($P = 5$ per cent) and 2·32 ($P = 5$ per cent) respectively.

In iron deficiency anaemia.—The per cent haemolysis recorded after 24 hours appeared to be lower than normal but this difference was not statistically significant. Similar results were obtained after 48 hours' incubation also. Some of the values recorded for iron deficiency anaemia were lower than the minimum value obtained for normal subjects.

Data on the packed cell volume show that, in normal bloods, the *PCV* increased after incubation for 24 hours and tended to come down to the original value after 48 hours. In Hb. E-thalassaemia, the *PCV* before and after incubation for 24 and 48 hours did not show much difference. In homozygous thalassaemia after incubation, there was a progressive increase in the *PCV* value over the initial value. Pattern obtained in thalassaemia trait was similar to that of homozygous thalassaemia. In Hb. E-trait, the *PCV* increased after 24 hours' incubation and this level was maintained even after 48 hours. In iron deficiency anaemia, the changes in the *PCV* values followed a normal pattern, the initial rise after 24 hours returning to original values after 48 hours' incubation. It appears that leptocytes in homozygous thalassaemia and thalassaemia trait continue to swell during 48 hours' incubation. Some red cells must have been destroyed during the process of

incubation as shown by the results of autohaemolysis. The progressive rise of the *PCV* values indicates that swelling of the cells was the dominating factor, masking the effect of haemolysis at least during 48 hours' incubation. In Hb. E-thalassaemia and Hb. E-trait, the swelling effect was not as prominent as in the previous two conditions.

*p*H of blood under the conditions of study did not show any significant difference among normal, Hb. E-thalassaemia, homozygous thalassaemia, Hb. E-trait, thalassaemia trait and iron deficiency anaemia indicating that the increased haemolysis is, in the present study, not related to changes in *p*H.

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STUDIES ON THE PHYSIOLOGY OF RICE

XVI. ROOT AND SHOOT GROWTH IN RELATION TO THE APPLICATION OF GROWTH REGULATORS AND CHANGES IN THE ENDOGENOUS FREE AUXIN CONTENTS *

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ABSTRACT

The root and shoot growth of the rice plant was studied after the application of growth regulators : IAA, NAA, MH and TIBA. These were applied by soaking seeds, growing the seedlings in solutions for six days and the plants in water culture with added regulators. During soaking, substance or substances are found diffusing out of the seeds which show inhibition of root growth.

The acceleration of flowering with high percentage of sterility of spikelets was noticed. All these regulators were found to have similar effects on flowering. The data on height, tiller and leaf number indicated that earliness was associated with the reduction of vegetative growth.

Bioassay of free auxin showed that endogenous auxin increased in different organs of the plants growing in nutrient solutions with regulators, maximum increase being noticed after IAA treatment.

The presence of high auxin level at the shoot apex at the time of transition was detected which would suggest that auxin content was not reduced prior to flowering but it accelerated flowering by the elongation of the shoot apex.

The number of lateral rootlets was considerably increased both in high and low concentrations of the regulators. Stimulation of root elongation was noticed in the treatment of seedlings for six days and in the plants grown in the nutrient solutions with the regulators. The external supply of the growth substances increased the free auxin content of root which stimulated both root elongation and the number. The endogenous auxin content of root was lower in NAA and MH than in IAA treatments.

An explanation for the effects of the different growth regulators on stimulation of root elongation and accumulation of free auxin in root has been offered.

INTRODUCTION

In recent years the physiology of flowering has received considerable attention. The problem has been approached on two aspects : (1) Attempts have been made to show flowering response in the non-induced plants with extracts added from photoinduced organs presumably with the idea of having

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a clue to the flower forming substance.   (2) Approach has been made to present evidence of flower initiation by the application of several growth regulators. The results obtained have been interpreted to show the identity of flowering substance with a particular compound.

Leopold (1958) has recently made a review of the problem of 'Auxin uses in the control of flowering and fruiting'.   The results from different workers indicate that behaviour of growth regulators vary with different plants.   The cause of this variation is presumably related with the nature of the native auxin in the plant.   The effect of external application would depend on the interaction between external auxin concentration and the age of the plant.

The flowering of rice induced by spraying with IAA and NAA was reported from this laboratory (Sircar and Kundu 1955).   An interesting feature is the elongation of the apex prior to flower initiation similar to that noted after photoperiodic induction.

It may also be mentioned that no work on the auxin relation of rice to root growth has been studied.   In view of the important relationship of auxin to root growth as recently reviewed by Burstrom (1953), Torrey (1956) and Åberg (1957), the scope of the work was extended to include a study of root growth in relation to the application of different growth regulators and the internal auxin concentration.   This has been approached by studying the root and shoot growth of rice plants from the early stage of seed soaking to the culture of the seedlings and the mature plants in nutrient solution with added growth regulators.

MATERIAL AND METHOD

The experiments have been carried out with pure line rice variety *Chinsurah Boro I* obtained from the Rice Research Station, Chinsurah, West Bengal.

The free auxin levels of the different organs of the plants in water culture were assayed by Root Inhibition Technique described previously (Sircar and Chakravarty 1957).   This method has been successfully adopted to show slight variations in the auxin level of the rice plant.   The root system, leaves, crown, stem base, stem and shoot apex were separated from a representative number of plants and auxin content determined separately for each organ.

EXPERIMENT 1.   SEED TREATMENT

Healthy seeds were treated with indole acetic acid (IAA), naphthalene acetic acid (NAA), maleic hydrazide (MH), and triiodo benzoic acid (TIBA) of four different concentrations, e.g. 1,000, 10, 0·1 and 0·001 mg. per litre, and seeds soaked in distilled water served as control.   100 unhusked seeds weighing 3·4 gm. were soaked in 100 ml. solution for 24 hours.

In order to determine whether growth substances are leached out by the seeds during soaking the following test was performed:—

Duplicate sets were taken for each of the concentrations. In one seeds were soaked for 24 hours and in the other no seed was soaked. In the distilled water control there were also two sets, one with seed and the other without seed. These solutions were then taken in 10 test tubes lined with filter paper, seedlings were inserted on the filter paper and after 48 hours root and shoot growth measured (Table 1A). It is interesting to note that the solution in which seeds were soaked showed greater root inhibition than the one without seeds. This experiment was also repeated with sprouted seeds as test materials in summer and the results are presented in Table 1B. Variations in the winter experiment with 0·1 and 0·001 mg. NAA and 0·001 mg. IAA per litre were noticeable but such differences are not seen in the summer. These are possibly related with the age of the seedlings used in two experiments. The sprouted seeds appear to be more adversely affected by different concentrations of the regulators than the seedlings. When seedlings were used, the effect of the regulators at 0·001 mg. concentration was increasing root growth after six days. The difference in the growth rate of seedlings of the control in summer and winter is due to temperature variations.

Thus having taken into consideration the root and shoot growth in the distilled water control for 48 hours as the normal growth of the seedling, it appears that the growth inhibition of root in leachings from seeds is due to the diffusion out of some substances from the seeds.

The seedlings in different concentrations show a general reduction in growth in comparison to normal growth in distilled water. But further reduction in growth when they are kept in leached solution would indicate that some inhibitory substance or substances are diffusing out from the seed. Even in distilled water used after soaking the seeds the root growth is reduced in comparison to normal growth in the control.

The inhibition effect is not so marked and regular in the shoot growth (Table 1), but it is regular and statistically significant in most of the root growth. Further work to determine the nature of the inhibitory substance excreted from seeds during soaking is now in progress.

Growth and flowering.—Seeds after 24 hours' soaking were thoroughly washed in water for five minutes and allowed to sprout for another 24 hours in petridishes lined with moist filter paper. On November 16, 1957, these were sown in pots and subsequent growth and flowering data were taken at intervals of 15 days. The final growth and flowering data are however only presented in Tables 2 and 3. In all the treatments there was early flowering with sterility of grains and the vegetative growth reduced.

TABLE 1

The inhibition of root and shoot growth by the leachings from the seeds during treatment

Experiment A: in winter (12-11-57) .. Initial length of roots = 18·95 ± 1·15
Initial length of shoots = 7·05 ± 0·45

Experiment B: in summer (10-5-58) .. Sprouted seeds.

Experiment No.	Treatment in mg./l.	IAA				NAA				MH				TIBA			
		X		Y		X		Y		X		Y		X		Y	
		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
A	1,000	21·80 ±1·55	8·50 ±1·00	24·18 ±2·42	11·40 ±1·20	20·20 ±1·33	8·60 ±0·86	21·70 ±1·20	7·40 ±1·20	21·18 ±1·09	15·80 ±0·48	26·66 ±0·66	15·90 ±1·04	19·10 ±1·60	8·30 ±0·72	26·90 ±1·70	8·50 ±0·78
	10	25·40 ±1·90	32·40 ±2·71	26·00 ±1·80	31·00 ±2·42	21·00 ±1·30	32·00 ±4·90	22·22 ±1·50	23·20 ±1·03	24·30 ±1·70	30·62 ±1·17	28·50 ±1·87	23·33 ±1·04	22·90 ±1·21	16·88 ±2·30	30·70 ±1·80	19·00 ±1·18
	0·1	32·66 ±4·30	22·77 ±0·96	44·00 ±3·50	21·70 ±1·05	24·18 ±3·51	30·09 ±2·93	22·10 ±1·90	25·00 ±1·79	34·60 ±3·90	21·60 ±0·45	41·70 ±0·31	20·80 ±0·80	25·80 ±1·60	21·20 ±1·25	31·80 ±2·70	20·10 ±1·65
	0·001	54·70 ±2·16	20·50 ±0·68	46·40 ±4·50	19·29 ±1·40	38·83 ±4·07	20·27 ±2·00	26·30 ±2·10	26·75 ±3·60	40·45 ±3·98	21·99 ±0·90	47·10 ±3·08	20·10 ±1·20	37·18 ±3·80	21·00 ±0·81	50·72 ±3·53	21·72 ±1·53
	Distilled water	51·55 ±2·70	20·44 ±0·81	56·00 ±1·70	19·66 ±0·84	—	—	—	—	—	—	—	—	—	—	—	—

B																
1,000	0	5·70 ±0·59	0	8·50 ±0·73	0	4·40 ±0·45	0	4·20 ±0·64	2·60 ±0·24	3·30 ±0·58	2·60 ±0·40	2·80 ±0·49	0	3·30 ±0·42	0	2·10 ±0·83
10	7·10 ±1·04	13·10 ±0·88	20·40 ±1·13	23·70 ±2·83	1·80 ±0·13	11·70 ±0·53	2·30 ±0·15	13·60 ±0·47	19·70 ±0·85	14·60 ±1·21	23·70 ±1·06	14·10 ±1·33	23·20 ±1·76	14·80 ±0·86	28·00 ±1·22	17·10 ±0·87
0·1	14·90 ±0·82	15·10 ±1·62	20·70 ±1·53	17·30 ±1·42	3·50 ±0·37	15·60 ±0·96	3·20 ±0·52	15·20 ±0·67	32·30 ±1·63	20·10 ±2·07	41·30 ±2·57	20·00 ±1·26	41·40 ±1·25	19·80 ±1·42	45·10 ±2·86	17·60 ±0·88
0·001	24·70 ±1·01	19·80 ±1·43	35·20 ±1·23	17·50 ±1·43	11·40 ±1·44	12·10 ±1·86	15·60 ±1·25	19·10 ±1·09	29·90 ±1·96	21·70 ±1·05	40·10 ±1·25	20·50 ±1·25	35·50 ±0·94	19·70 ±1·22	58·20 ±1·78	19·80 ±1·20
Distilled water	48·60 ±1·94	25·30 ±1·22	63·80 ±1·32	26·60 ±1·08	—	—	—	—	—	—	—	—	—	—	—	—

X = Growth of seedlings in mm. for 48 hours in solutions used for seed soaking.

Y = Growth of seedlings in mm. for 48 hours in solutions not used for seed soaking.

TABLE 2

Final reading of height, tiller and leaf number of rice variety Chinsurah Boro I after seed treatment (Experiment)

(Average of 10 plants)

Concentration in mg/l.	IAA			NAA			MH			TIBA		
	Height	Tiller number	Leaf number	Height	Tiller number	Leaf number	Height	Tiller number	Leaf number	Height	Tiller number	Leaf number
1,000	55·71 ±1·67	5·20 ±0·46	14·30 ±0·15	47·19 ±1·71	5·00 ±0·49	14·20 ±0·29	55·71 ±1·67	7·00 ±0·39	14·00 ±0·21	Did not germinate		
10	50·50 ±1·19	4·70 ±0·33	14·40 ±0·22	51·99 ±1·31	5·60 ±0·40	14·30 ±0·15	56·99 ±1·55	7·00 ±0·33	14·40 ±0·22	51·68 ±1·50	6·60 ±0·65	14·30 ±0·26
0·1	50·02 ±1·55	6·20 ±0·49	14·20 ±0·20	60·53 ±1·88	7·50 ±0·50	14·20 ±0·20	50·71 ±2·10	8·30 ±0·47	14·60 ±0·26	51·38 ±1·00	7·40 ±0·49	14·50 ±0·26
0·001	51·48 ±1·83	7·30 ±0·44	14·40 ±0·16	55·63 ±1·78	4·80 ±0·40	41·30 ±0·21	53·12 ±1·47	9·20 ±0·59	14·50 ±0·37	50·59 ±1·63	7·60 ±0·56	14·30 ±0·26
Control (soaked in distilled water)	56·74 ±1·51	8·30 ±0·53	15·70 ±0·21									

TABLE 3

Flowering and percentage of grains in the main shoot of Chinsurah Boro I after seed treatment

Sowing date—16-11-57

(Average of 10 plants)

Concentration in mg./l.	IAA		NAA		MH		TIBA	
	Number of days for ear emergence	% of fertile grains per ear	Number of days for ear emergence	% of fertile grains per ear	Number of days for ear emergence	% of fertile grains per ear	Number of days for ear emergence	% of fertile grains per ear
1,000	136·70 ± 2·40	47·22	135·80 ± 2·74	53·42	130·50 ± 1·20	42·44	Did not germinate	
10	136·60 ± 2·45	46·65	131·90 ± 1·84	55·10	127·50 ± 1·60	54·52	131·70 ± 1·05	57·88
0·1	137·00 ± 2·30	47·71	130·10 ± 2·55	44·22	131·10 ± 2·28	52·37	130·20 ± 1·76	44·68
0·001	134·20 ± 1·10	50·46	135·20 ± 2·81	46·61	126·10 ± 1·70	47·24	131·90 ± 2·84	47·24
Control (soaked in distilled water)	141·80 ± 2·61	63·03						

. EXPERIMENT 2.   SEEDLING TREATMENT

This experiment was performed to study root and shoot growth of sprouted rice seedlings in different concentrations of the regulators in test tubes for a period of six days and subsequently transplanted in pots.

The concentrations of IAA, NAA and MH used are 1,000, 10, 0·1 and 0·001 mg. per litre. The sprouted grains were placed on the brim of filter paper lining the culture tubes. The experimental plants were grown under normal condition for six days and root and shoot growth measured (Table 4). Subsequently seedlings were transplanted to pots. There were two controls:— One with the seedlings grown in distilled water, while the second control was maintained by growing the sprouted seeds in pots instead of test tubes. The object of keeping control II is to compare the effects of distilled water treatment in test tubes with the normal seedlings grown in the field.

Growth and flowering.—The growth reading after six days shows that the higher the concentration of the regulators the greater is the inhibition of root and shoot growth whereas in lower concentration (0·001 mg./l.) the root growth is increased (Table 4 and Fig. 1).

The shoot growth was measured at intervals of 15 days till flowering. The data for final height, tillering and leaf number presented (Table 5) show a general reduction as compared with the control but the differences are not statistically significant.   In treatments with IAA, NAA and MH an acceleration of flowering with reduction of fertile grains is observed which is statistically significant in some cases (Table 6).

EXPERIMENT 3.   CULTURE OF RICE SEEDLINGS IN NUTRIENT SOLUTION WITH GROWTH REGULATORS AND THE ENDOGENOUS AUXIN LEVELS OF DIFFERENT ORGANS

Seeds were treated with 0·02 per cent formalin for 5 to 10 minutes, thoroughly washed and allowed to sprout overnight in petridishes lined with moist filter paper.

The sprouted seedlings were grown in series of test tubes containing distilled water.   After three days seedlings were transferred to culture bottles of one litre capacity three-fourths of which were filled with nutrient solutions of Gregory and Purvis (1938) and Purvis (1944).   The quantity of iron was twice the amount used by Purvis and Gregory as rice seedlings are known to require a large amount.   Different concentrations, 10, 1, 0·1, 0·01 and 0·001 mg. per litre of IAA, NAA and MH, were added in the nutrient solutions.   Control plants were grown in water culture without added growth regulators.   The bottles covered with black paper and the mouth plugged with non-absorbent cotton were kept in the pot culture house of the department (Fig. 2).   Aeration with the help of a hand pump was made at frequent intervals.   Freshly

Distilled water

0·001 mg./l.

0·01 mg./l.

0·1 mg./l.

1 mg./l.

10 mg./l.

100 mg./l.

1,000 mg./l.
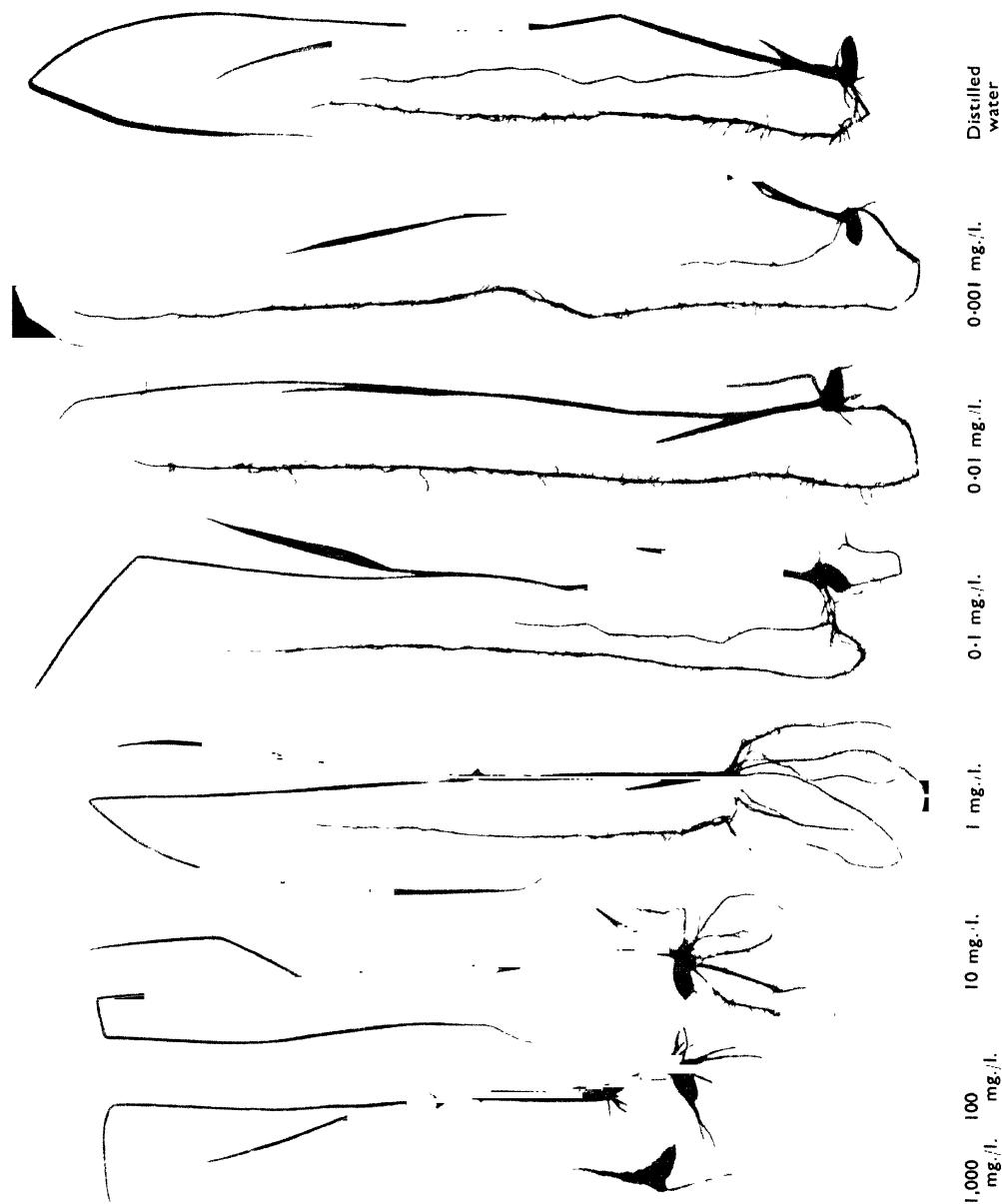
TABLE 4

Growth of seedlings in test tubes after six days' treatment with regulators

(Average of 6–10 plants)

Concentration in mg./l.	IAA		NAA		MH	
	Length of the root in cm.	Length of the shoot in cm.	Length of the root in cm.	Length of the shoot in cm.	Length of the root in cm.	Length of the shoot in cm.
1,000	0·61±0·15	2·60±0·22	0·58±0·26	1·15±0·13	0·20±0·04	1·33±0·03
10	2·53±0·34	5·09±0·58	1·65±0·09	3·08±0·13	2·83±0·10	2·65±0·09
0·1	9·08±0·96	9·39±0·66	4·30±0·48	5·73±0·32	10·48±0·44	9·99±1·02
0·001	14·46±0·87	10·76±1·17	11·70±0·84	11·05±0·31	15·70±0·35	9·70±0·31
Control (distilled water)	11·43±0·37	12·21±0·26				

TABLE 5

Height, tiller and leaf number of the 120 days' old plants after transplantation in pots of the treated seedlings for six days in test tubes

(Average of 10 plants)

Concentration in mg./l.	IAA			NAA			MH		
	Height	Tiller number	Leaf number	Height	Tiller number	Leaf number	Height	Tiller number	Leaf number
1,000	Did not survive after transplanting to pots								
10	54·94±2·87	7·70±0·36	14·10±0·23	50·81±1·18	7·60±0·40	14·30±0·15	Did not survive after transplanting to pots		
0·1	52·29±1·49	8·50±0·47	14·30±0·26	54·74±2·39	6·00±0·21	13·70±0·21	50·02±1·29	7·10±0·48	13·60±0·16
0·001	54·49±2·50	6·90±0·60	13·70±0·15	51·57±1·55	5·20±0·38	14·00±0·20	51·96±1·44	6·20±0·38	14·30±0·36
Control I (distilled water)	58·80±1·26	9·90±0·60	15·60±0·16						
Control II (sown in pots just after sprouting)	56·88±0·99	8·80±0·24	15·40±0·16						

TABLE 6

Flowering and percentage of grains in the main shoot after treated seeds transplanted to pots on 16-11-57

(Average of 10 plants)

Concentration in mg./l.	IAA		NAA		MH	
	Number of days for ear emergence	% of fertile grains per ear	Number of days for ear emergence	% of fertile grains per ear	Number of days for ear emergence	% of fertile grains per ear
1,000	Did not survive after transplanting to pots					
10	130·10 ± 2·76	37·46	138·60 ± 1·04	43·92	Did not survive after transplanting to pots	
0·1	132·10 ± 2·13	46·79	129·20 ± 2·09	42·54	128·70 ± 2·59	46·56
0·001	128·10 ± 1·40	47·66	132·00 ± 2·44	46·03	136·90 ± 2·03	41·20
Control I (distilled water)	146·50 ± 2·05	48·26				
Control II (sown to pots just after sprouting)	144·80 ± 1·69	53·38				

prepared nutrient solutions and the growth hormones were changed after every seven days.   In order to avoid the rise of temperature in the water culture bottles in the summer season it was found convenient to keep them partly buried in the soil (Fig. 2).   This made the culture plants show normal growth (Figs. 3 and 4).   Root and shoot growth of the plants was determined at intervals of 10, 28 and 76 days.   On these days the auxin contents of the various parts were assayed by root inhibition technique.

Root growth.—Higher concentrations of IAA show toxic effects on root growth.   In 1 mg./l. of IAA the toxic effect is noticed at 10 days but afterwards it recovers, and more or less attains the same length as in other lower concentrations.   There was a significant increase in root length in 0·1 to 0·001 mg./l. of IAA (Table 7 and Figs. 5 and 8).   The data for the number of roots at different ages indicate a considerable increase in treatments ranging from 1 to 0·001 mg./l. of IAA which are also statistically significant (Table 7).

In 1 mg./l. of NAA the toxic effect was prominent, the plants survived for 76 days and then died before flowering.   The toxic effect was also noticed in 0·1 mg./l. up to 10 days but in later stages the plants recovered while in 0·001 mg./l. there was a significant increase in the root length after 28 and 76 days (Table 7 and Figs. 6 and 8).   The number of roots increased progressively in concentrations varying from 1 to 0·001 mg./l. (Table 7).

Unlike the treatments with IAA and NAA the toxic effect on the root growth is not so prominent after adding MH in the nutrient solution (Table 7). In higher concentrations adverse effect on elongation is noticeable while lower concentrations stimulate root elongation (Figs. 7 and 8).   The number of roots increased after 13 days in treatments with MH ranging from 1 to 0·001 mg./l. and in the later period the number is considerably increased at 0·001 mg./l. (Table 7).

Auxin level of root.—In the initial stage, before transferring the three-day old seedlings to the nutrient solution the internal auxin content is very low.   With external supply of IAA the total free auxin and its concentration in the root system is increased (Table 8).   The increase after the application of NAA is not to the same extent as in the IAA treatment.   The auxin level in treatments with MH is less than that noted with IAA and NAA (Table 8). It appears that in lower concentration of IAA auxin content generally rises with growth but is reduced after 28 days and a subsequent rise noticed at the end of 76 days.   The total auxin and concentration in root after 76 days is less in 1 mg./l. of IAA than in other treatments.   This is presumably related with high auxin translocation in the stem tissue and apex (Tables 8 and 12). The same picture is also noticed in the control root and stem.   It is interesting to note that a large number of roots noticed in the earlier stage in 1 mg./l. of IAA are associated with high auxin concentration (Tables 7 and 8).   In the lower concentration of NAA the auxin level decreases after 10 days which

FIG. 2.   General view of the plants in pot culture house.
FIG. 3.   Plants showing ear emergence.
FIG. 4.   Plants after grain formation.
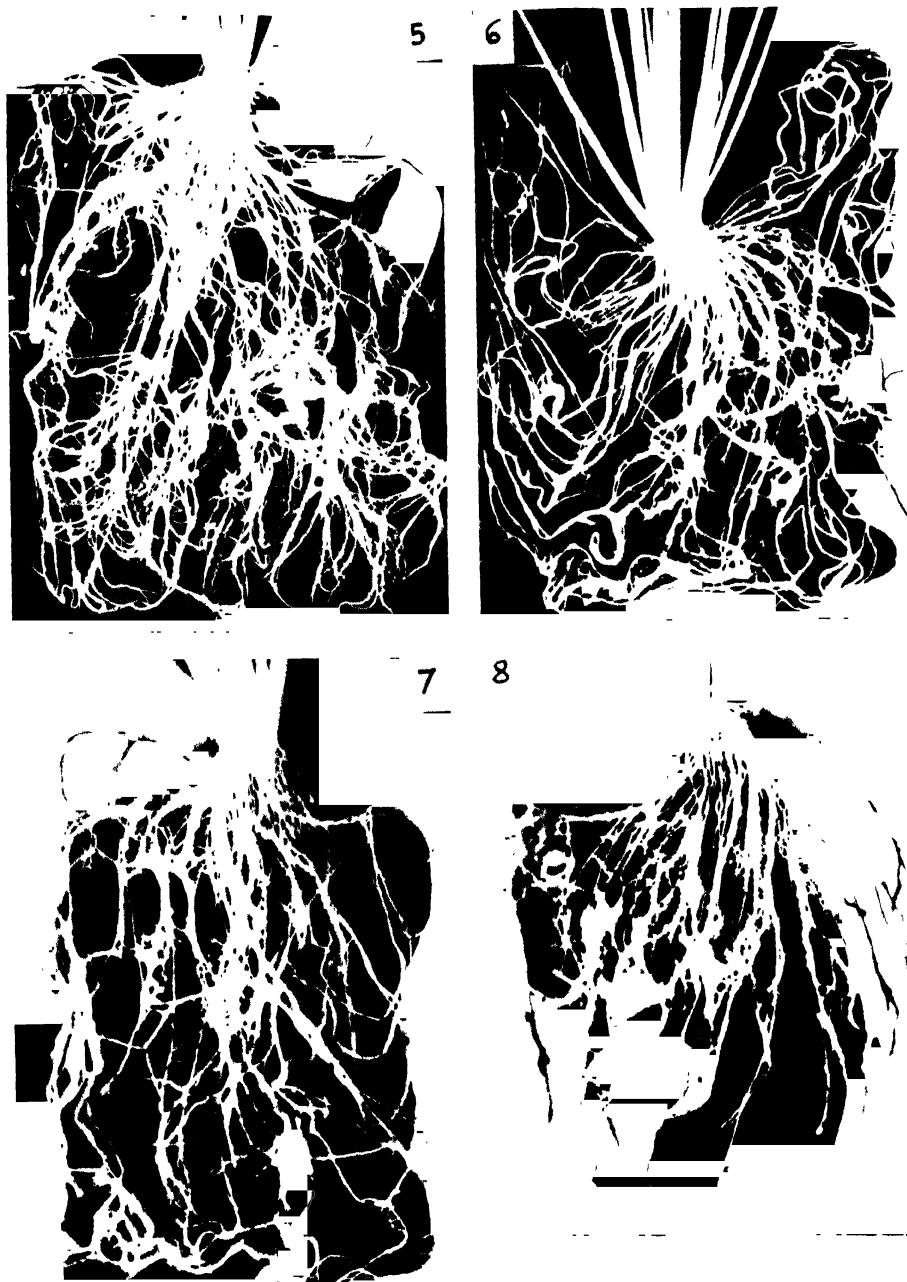
FIG. 5.   Root growth in 0·001 mg./l. IAA after 76 days.
FIG. 6.   Root growth in 0·001 mg./l. NAA after 76 days.
FIG. 7.   Root growth in 0·001 mg./l. MH after 76 days.
FIG. 8.   Root growth in control (nutrient solution) after 76 days.

TABLE 7

Root growth in nutrient solutions with added growth regulators

(Average of 6–10 plants)

Concentration in mg./l.	Age of the plants in days	IAA		NAA		MH	
		Length of the longest roots in cm.	Number of roots	Length of the longest roots in cm.	Number of roots	Length of the longest roots in cm.	Number of roots
Initial (distilled water)	3	5·34 ± 0·17	—	5·34 ± 0·17	—	5·34 ± 0·17	—
10	10	8·90 ± 0·22	7·50 ± 0·34	6·50 ± 0·03	4·00 ± 0·36	11·25 ± 0·87	4·16 ± 0·31
	28	6·60 ± 0·22	12·10 ± 0·47	6·90 ± 0·07	2·30 ± 0·20	11·95 ± 0·21	7·10 ± 0·54
	76	—Did not survive after one month		Did not survive after one month		Did not survive after one month	
1	10	10·57 ± 0·26	14·10 ± 0·31	6·65 ± 0·02	5·50 ± 0·62	12·47 ± 0·26	5·66 ± 0·42
	28	25·90 ± 0·37	21·83 ± 0·70	7·60 ± 0·19	4·66 ± 0·32	20·27 ± 0·24	12·00 ± 0·81
	76	34·00 ± 0·33	100·00 ± 1·04	10·60 ± 0·19	17·50 ± 0·71	Did not survive after one month	
0·1	10	20·70 ± 0·43	12·00 ± 0·25	7·40 ± 0·11	5·66 ± 0·32	20·00 ± 0·15	6·6 6 ± 0·42
	28	24·60 ± 0·29	18·66 ± 0·33	10·88 ± 0·12	11·00 ± 0·43	22·85 ± 0·22	13·16 ± 0·60
	76	36·00 ± 0·06	103·10 ± 0·65	28·00 ± 0·25	70·00 ± 0·57	29·10 ± 0·15	60·00 ± 0·36
0·01	10	22·20 ± 0·29	6·10 ± 0·41	12·73 ± 0·57	6·66 ± 0·32	23·60 ± 0·30	9·50 ± 0·34
	28	25·80 ± 0·22	15·50 ± 0·42	20·85 ± 0·22	14·00 ± 0·77	25·40 ± 0·15	13·50 ± 0·64
	76	35·00 ± 0·09	105·30 ± 0·61	36·30 ± 0·27	100·00 ± 0·57	30·00 ± 0·17	70·00 ± 0·57
0·001	10	27·80 ± 0·36	6·16 ± 0·31	12·70 ± 0·21	8·16 ± 0·47	24·10 ± 0·50	9·55 ± 0·44
	28	29·30 ± 0·23	16·16 ± 0·19	25·50 ± 0·30	15·66 ± 0·42	30·10 ± 0·73	15·33 ± 0·60
	76	38·50 ± 0·30	108·10 ± 0·47	35·00 ± 0·18	107·00 ± 0·76	31·80 ± 0·03	90·00 ± 0·45
Control (nutrient solution only)	10	18·33 ± 0·20	4·71 ± 0·42				
	28	21·76 ± 0·27	10·99 ± 0·40				
	76	27·00 ± 0·31	50·00 ± 0·50				

TABLE 8

Free auxin content of root system growing in nutrient solution with added regulators

		IAA			NAA			MH		
Concentration in mg./l.	Age of the plants in days	Fresh weight in gm.	Total free auxin per root system (μg. IAA Eq.)	Free auxin concentration on per gm. fresh weight (μg. IAA Eq.)	Fresh weight in gm.	Total free auxin per root system (μg. IAA Eq.)	Free auxin concentration on per gm. fresh weight (μg. IAA Eq.)	Fresh weight in gm.	Total free auxin per root system (μg. IAA Eq.)	Free auxin concentration on per mg. fresh weight (μg. IAA Eq.)
Initial 10	3	0·040	0·003	0·070						
	10	0·115	0·542	4·715	0·052	0·090	1·742	0·141	0·101	0·721
	28	0·197	0·216	1·110	0·115	0·017	0·154	0·147	0·094	0·641
	76				Did not survive after one month			Did not survive after one month		
1	10	0·120	0·426	3·545	0·108	0·140	1·304	0·192	0·197	1·027
	28	0·418	0·482	1·152	0·149	0·250	1·681	0·232	0·111	0·482
	76	4·200	1·115	0·265	0·210	0·039	0·189	Did not survive after one month		
0·1	10	0·132	0·316	2·390	0·105	0·185	1·765	0·195	0·084	0·431
	28	0·395	0·452	1·143	0·280	0·037	0·134	0·375	0·265	0·708
	76	4·500	8·890	1·975	3·050	0·295	2·063	4·000	0·008	0·002
0·01	10	0·195	0·261	1·340	0·115	0·254	2·213	0·199	0·024	0·123
	28	0·398	0·333	0·836	0·285	0·027	0·097	0·381	0·062	0·164
	76	4·700	9·975	2·122	4·500	15·850	3·502	4·100	0·111	0·027
0·001	10	0·198	0·199	1·005	0·110	0·183	1·675	0·201	0·012	0·059
	28	0·400	0·316	0·788	0·290	0·018	0·064	0·397	0·049	0·124
	76	4·900	5·115	1·043	4·700	3·548	0·754	4·500	0·223	0·049
Control (nutrient solution only)	10	0·196	0·055	0·282						
	28	0·352	0·042	0·119						
	76	3·500	0·039	0·011						

rises again at the end of 76 days, at this stage it is even higher than in the IAA treatments.   In general the trend of auxin content in treatments with NAA appears to be the same as noticed in IAA.   In the lower concentration of MH a slight rise after 28 days and subsequent fall after 76 days is noticed.   On the other hand there is a gradual fall from 10 to 76 days in the control series. In some lower concentrations of MH the native auxin was found to be lower than the control.

Shoot growth.—The toxic effect of higher dosages of the regulators is evident from the survival of the plants for a period of one month only, while in lower concentrations plants maintained normal, healthy growth.   At these levels of IAA, NAA and MH there is no significant increase in leaf number and tillering (Table 9).   A slight acceleration of flowering is noticed but the fertility of the spikelets is reduced in comparison to the control (Table 13).

AUXIN LEVELS OF CROWN, LEAVES, STEM AND SHOOT APEX

From the distribution of auxin in crown, stem, leaf and shoot apex in IAA treatments an overall picture may be visualized (Tables 10 to 12).   Auxin appears to decrease in crown tissue from 28 to 76 days.   This disappearance is presumably related to its utilization in root growth and for upward translocation to stem, while the high level in the shoot apex results from the mobilization of auxin from the leaves.   In the NAA treatments the amount of native auxin available in crown, stem tissue and shoot apex is higher than in the control.   The auxin level in the leaves after 76 days' growth appears to be unusually high in NAA treatments.   Unlike IAA and NAA the level in the stem after MH treatment is generally lower than the control.   In the shoot apex it is still lower than the stem.   Although high auxin content is noticed in the leaves of MH treatment but in comparison to NAA and IAA treatments its translocation to stem and shoot apex is rather restricted.   In the control, high auxin level in the leaf and stem and less transport to the apex is apparent.

DISCUSSION

The application of growth regulators to rice plants at different stages of growth shows interesting results.   In a previous study (Sircar and Kundu 1955) the acceleration of flowering and a high per cent of sterility in a photosensitive variety by spraying with IAA and NAA was reported.   Misra and Sahu (1957, 1958) confirmed early flowering in late winter varieties by seed soaking followed by spray with these regulators but in several other varieties the results were inconsistent.   Sarkar (1958) while studying the interaction between photoperiodism and auxin in three differently photosensitive varieties reported early flowering with sterility of grains.   Results presented in

TABLE 9

Shoot growth (height, tiller and leaf number) of the plants growing in culture solution with added regulators

(Average of 6–10 plants)

Concentration in mg./l.	Age of the plants in days	IAA			NAA			MH		
		Length of the shoots in cm.	Number of tillers	Number of leaves	Length of the shoots in cm.	Number of tillers	Number of leaves	Length of the shoots in cm.	Number of tillers	Number of leaves
Initial (distilled water)	3	2·35 ± 0·02	—	Coleoptile only	—	—	—	—	—	—
10	10	10·35 ± 0·18		2·0 ± 0·0	6·30 ± 0·35		2·0 ± 0·25	13·80 ± 0·29		2·0 ± 0·25
	28	15·90 ± 0·49		3·5 ± 0·23	9·90 ± 0·11		2·3 ± 0·20	17·35 ± 0·12		3·8 ± 0·40
	76				Did not survive after one month			Did not survive after one month		
1	10	23·25 ± 0·30		2·0 ± 0·25	14·02 ± 0·08		2·0 ± 0·36	16·80 ± 0·45		2·0 ± 0·36
	28	26·62 ± 0·51		5·3 ± 0·93	14·75 ± 0·22		2·5 ± 0·22	24·45 ± 0·23		4·6 ± 0·32
	76	54·00 ± 0·14	3·3 ± 0·30	8·3 ± 0·32	17·70 ± 0·15	Nil	5·8 ± 0·47			
0·1	10	20·37 ± 0·35		2·3 ± 0·20	18·20 ± 0·77		2·3 ± 0·20	21·40 ± 0·22		2·5 ± 0·22
	28	22·30 ± 0·28		6·0 ± 0·36	25·00 ± 0·69		4·8 ± 0·30	25·10 ± 0·30		6·3 ± 0·50
	76	51·00 ± 0·23	4·5 ± 0·20	10·6 ± 0·44	40·50 ± 0·28	3·1 ± 0·30	11·3 ± 0·32	50·00 ± 0·14	5·1 ± 0·30	12·3 ± 0·20
0·01	10	20·70 ± 0·43		2·5 ± 0·22	18·30 ± 0·22		2·5 ± 0·22	20·50 ± 1·14		2·5 ± 0·22
	28	25·70 ± 0·51		6·8 ± 0·50	24·90 ± 0·79		5·8 ± 0·30	21·80 ± 0·13		6·8 ± 1·50
	76	52·00 ± 0·24	4·6 ± 0·20	12·8 ± 0·30	60·50 ± 0·23	4·5 ± 0·20	11·8 ± 0·40	63·00 ± 0·37	6·6 ± 0·32	14·1 ± 0·30
0·001	10	20·50 ± 0·57		2·6 ± 0·21	19·12 ± 1·05		2·5 ± 0·22	21·20 ± 0·32		2·6 ± 0·21
	28	30·00 ± 1·26		7·1 ± 0·30	26·00 ± 0·35		5·5 ± 0·42	26·10 ± 0·37		6·6 ± 1·10
	76	53·50 ± 0·17	6·1 ± 0·30	13·0 ± 0·44	60·80 ± 0·35	5·8 ± 0·30	12·1 ± 0·30	62·00 ± 0·24	6·6 ± 0·36	13·5 ± 0·44
Control (nutrient solution only)	10	19·30 ± 0·15		2·5 ± 0·21						
	28	23·99 ± 0·26		6·0 ± 0·38						
	76	59·60 ± 0·15	5·4 ± 0·42	13·2 ± 0·49						

TABLE 10

Free auxin content of leaves (shoots) after addition of IAA, NAA and MH in the culture solution

Concentration in mg./l.	Age of the plants in days	IAA			NAA			MH		
		Fresh weight in gm.	Total free auxin per plant (µg. IAA Eq.)	Free auxin concentration on per gm. fresh weight (µg. IAA Eq.)	Fresh weight in gm.	Total free auxin per plant (µg. IAA Eq.)	Free auxin concentration on per gm. fresh weight (µg. IAA Eq.)	Fresh weight in gm.	Total free auxin per plant (µg. IAA Eq.)	Free auxin concentration on per gm. fresh weight (µg. IAA Eq.)
Initial 10	3	0·039	0·001	0·0358						
	10	0·121	1·744	14·411	—	—	—	0·152	5·363	35·281
	28	0·240	0·053	0·223				0·200	0·272	1·363
	76	— Did not survive after one month —								
1	10	0·205	0·270	1·320	0·125	0·041	0·324	0·197	0·232	1·182
	28	0·327	3·921	11·992	0·157	0·167	1·067	0·305	0·054	0·179
	76	1·510	0·199	0·132	0·275	0·638	2·321	— Did not survive after one month —		
0·1	10	0·195	0·114	0·587	0·150	0·356	2·308	0·197	0·257	1·305
	28	0·305	0·910	3·018	0·312	0·140	0·451	0·415	0·058	0·141
	76	1·700	0·251	0·147	1·700	50·120	29·482	0·914	9·975	10·913
0·01	10	0·204	0·084	0·412	0·156	0·323	2·076	0·202	0·185	0·915
	28	0·360	0·555	1·543	0·375	0·207	0·553	0·405	0·012	0·031
	76	2·000	5·235	2·617	1·500	25·120	16·746	1·500	11·220	7·480
0·001	10	0·212	0·055	0·261	0·157	0·016	0·103	0·200	0·167	0·838
	28	0·340	0·310	0·912	0·397	0·269	0·677	0·422	0·035	0·083
	76	2·300	11·995	5·215	3·400	22·390	6·585	2·500	12·590	5·036
Control (nutrient solution only)	10	0·200	0·031	0·155						
	28	0·310	0·060	0·190						
	76	2·700	57·540	21·311						

TABLE 11

Free auxin content of crown and stem base after application of IAA, NAA and MH in the nutrient solution

Concentration in mg./l.	Nature of tissue	Age of the plants in days	IAA Fresh weights in gm.	IAA Total free auxin per plant (μg. IAA Eq.)	IAA Free auxin concentration on per gm. fresh weight (μg. IAA Eq.)	NAA Fresh weights in gm.	NAA Total free auxin per plant (μg. IAA Eq.)	NAA Free auxin concentration on per gm. fresh weight (μg. IAA Eq.)	MH Fresh weights in gm.	MH Total free auxin per plant (μg. IAA Eq.)	MH Free auxin concentration on per gm. fresh weight (μg. IAA Eq.)
Initial 10	(Crown)	3	0·010	0·0006	0·062						
	,,	10	0·047	0·223	4·749	0·015	0·021	1·424	0·047	0·189	4·025
	,,	28	0·117	0·025	0·219	0·112	0·003	0·033	0·151	0·020	0·133
	(Stem base)	76	Did not survive after one month								
1	(Crown)	10	0·063	0·062	0·964	0·020	0·023	1·185	0·051	0·151	2·967
	,,	28	0·124	0·504	4·064	0·110	0·785	7·143	0·165	0·025	0·152
	(Stem base)	76	0·510	0·0001	0·0002	0·111	0·239	2·158	Did not survive after one month		
0·1	(Crown)	10	0·095	0·054	0·572	0·035	0·115	3·303	0·055	0·123	2·245
	,,	28	0·197	0·252	1·280	0·157	0·045	0·292	0·197	0·060	0·309
	(Stem base)	76	0·597	0·006	0·010	0·675	0·289	0·428	0·301	0·004	0·009
0·01	(Crown)	10	0·098	0·045	0·466	0·025	0·053	2·144	0·060	0·018	0·309
	,,	28	0·192	0·052	0·272	0·195	0·054	0·279	0·206	0·005	0·027
	(Stem base)	76	0·525	0·005	0·010	0·695	0·472	0·679	0·450	0·002	0·005
0·001	(Crown)	10	0·098	0·013	0·135	0·025	0·047	1·904	0·065	0·014	0·221
	,,	28	0·230	0·045	0·199	0·255	0·048	0·191	0·257	0·003	0·015
	(Stem base)	76	0·576	0·015	0·026	1·500	0·183	0·122	0·550	0·210	0·382
Control (nutrient solution only)	(Crown)	10	0·095	0·003	0·039						
	,,	28	0·230	0·025	0·108						
	(Stem base)	76	0·575	0·014	0·025						

TABLE 12

Free auxin content of stem and shoot apex of 76 days' old plants growing in nutrient solution with IAA, NAA and MH

Concentration in mg./l.	Nature of tissue	IAA			NAA			MH		
		Fresh weights in gm.	Total free auxin per plant (µg. IAA Eq.)	Free auxin concentration on per gm. fresh weight (µg. IAA Eq.)	Fresh weights in gm.	Total free auxin per plant (µg. IAA Eq.)	Free auxin concentration on per gm. fresh weight (µg. IAA Eq.)	Fresh weights in gm.	Total free auxin per plant (µg. IAA Eq.)	Free auxin concentration on per gm. fresh weight (µg. IAA Eq.)
10	Stem	Did not survive after one month						Did not survive after one month		
1	,,	0.446	5.033	11.271	Merely survived up to 76 days but no stem tissue developed					
0.1	,,	1.125	0.726	0.646	0.940	9.432	10.345	0.602	0.079	0.131
0.01	,,	0.910	0.182	0.200	0.818	8.135	9.931	1.150	0.133	0.115
0.001	,,	0.710	0.074	0.104	1.000	5.610	5.610	0.950	0.169	0.178
Control (nutrient solution only)	Stem	0.685	4.372	6.382						
10	Shoot Apex	0.305	12.032	39.451	Did not survive after one month			Did not survive after one month		
1	,,	0.300	5.647	18.825	Merely survived up to 76 days without producing developing shoot apex					
0.1	,,				0.210	0.903	4.300	0.305	Below 0.00001 mg./l.	Below 0.00001 mg./l.
0.01	,,	0.310	5.375	17.340	0.245	5.952	24.297	0.265	0.002	0.010
0.001	,,	0.260	3.255	12.524	0.285	3.627	12.726	0.275	0.086	0.319
Control (nutrient solution only)	Shoot Apex	0.290	0.770	2.644						

TABLE 13

Flowering and percentage of fertile grains in the main shoot of the plants growing in culture solution with added regulators

Average of 10 plants

Concentration in mg./l.	IAA		NAA		MH	
	Days for ear emergence	% of fertile grains per ear	Days for ear emergence	% of fertile grains per ear	Days for ear emergence	% of fertile grains per ear
10	Did not survive till ear emergence		Did not survive till ear emergence		Did not survive till ear emergence	
1	99·41 ± 0·85	66·46	Did not survive till ear emergence		Did not survive till ear emergence	
0·1	93·25 ± 0·51	70·26	97·00 ± 0·60	76·75	97·12 ± 0·77	40·69
0·01	98·06 ± 0·93	61·51	98·93 ± 0·86	68·40	97·87 ± 0·55	46·30
0·001	92·68 ± 0·97	65·68	101·06 ± 0·83	73·67	99·56 ± 0·56	43·82
Control (nutrient solution only)	103·50 ± 0·93	75·30				

this paper show further that earliness could as well be induced in photoinsensitive variety by different methods of application (Tables 3, 6 and 13). The degree of earliness is greater in treatments with seed soaking and feeding the seedlings for 6 days than in culturing the plants in nutrient solutions with added regulators. It appears that all these growth regulators IAA, NAA, MH and TIBA behave in the same way but earliness is greater in TIBA and MH treatments. Reduction in height, tiller, leaf number and fertility of spikelets is associated with earliness as noticed in previous experiments with photoperiodic induction (Sircar and Parija 1949).

The induction of flower initiation by auxin application is still an open question. Audus (1953) stated that auxin has correlated influence on growth behaviour which ultimately leads to earlier flower formation. Another school of thought considers that flowering is induced by the lowering of auxin content. The latter observation is made from the effect of anti-auxin like TIBA and MH which is supposed to lower or inhibit the auxin level thereby ensuring early flowering. The experiments reported here show the effects of auxins and anti-auxins are more or less alike. It may therefore be suggested that the question of lowering or inhibiting the natural auxin does not arise in the rice plant showing acceleration of flowering by treatments with these regulators. On the contrary the auxin or the anti-auxin presumably induces early flowering by restricting vegetative growth, as indicated by tiller and leaf number. It has been stated by Audus that chemical treatments which reduce vegetative growth or arrest or kill the apical meristem induce early flowering. Previous photoperiodic experiments with rice varieties have shown that early flowering is associated with reduction of vegetative growth (Sircar and Parija 1949). In the present experiments the effects of the growth regulators have been reduction of vegetative growth from the very early stage which is later reflected in small but significant acceleration of flowering (Tables 2, 3, 5, 6, 9 and 13).

Sircar and Kundu (1955) have shown that soon after the application of auxins by spray elongation of the shoot apex and the formation of flowers are noticed. This obviously means that early flowering through auxin application is due to the effects on the vegetative growth as indicated in the elongation of the apex. Similarly it may be argued that treatments of seedlings with the chemicals for 6 days induce early flowering (Table 6) but no sign of flower initiation in the apex at the end of the treatment; only small changes in the dimension of the apex is visible (Kundu 1959).

Estimations of auxins were made at three stages from the plants growing in nutrient solutions with growth regulators. Distribution of auxin in crown, stem, shoot apex and leaves indicates a fall in crown tissue from 28 to 76 days. But in the leaves it gradually rises from 10 to 76 days and is always less than the control. This disappearance from crown tissue at 76 days is either related

to its utilization in root growth or translocation to stem and shoot apex which becomes elongated. The high auxin level in the shoot apex at this stage is also evident. The increased auxin content in the leaves of the treated plants after 76 days may also contribute to the auxin level of the shoot apex. Thus the high auxin level in the shoot apex is presumably accounted for from these two additional sources. On the other hand, the auxin level in the leaves and stem in the control plants is high indicating considerably less transport to the shoot apex. It is interesting to note that the endogenous auxin level in the leaves after MH treatment is high but the movement to stem and shoot apex is restricted.

In IAA treatment auxin is translocated more rapidly and in a larger quantity to shoot apex while in other cases transport up to stem is prominent. The rapid transport of IAA in treated plants is possibly due to more IAA content. Auxin was estimated from the apex after 76 days when the plants were at the transitional stage. The presence of high auxin at the shoot apex at the time of transition would obviously mean that the application of growth regulators does not reduce the auxin content prior to flowering which Bonner and Thurlow (1949) thought to be auxin action on the promotion of flowering.

The application of different concentrations of regulators does not show consistent increase in the shoot growth. At higher concentration toxic effect on root and shoot growth has been shown and the plants did not survive, while in the lower concentration the plants grew to maturity. The growth of the root in length is restricted at concentrations 10 and 1 mg./l. but the number of rootlets is considerably increased. In lower concentration stimulation of root growth both in number and length is very apparent (Figs. 5 to 8). Lower concentration of IAA, 0·001 mg./l., shows small increase in height after 28 days but at later stages the acceleration is not maintained (Table 9). The treatments with MH tend to increase the height of the plants while no significant difference is noticed in tiller and leaf number. The overall picture appears to be that there is no prospect of increasing the shoot growth of the rice plant by the application of growth regulators; on the contrary general reduction of vegetative growth is evident. Similar results with several other cereals have also been reviewed by Audus (1953) and Leopold (1955). It is interesting to note the auxin effect on the root growth of the rice plant. The relation of auxin to root growth by cell multiplication and cell elongation has been studied by several workers. There has been no question of the auxin effect on the cell multiplication as the phenomenon of rooting by applied auxin is an observed fact. But the relation of cell elongation of roots to applied auxin has been questioned by Åberg (1957). He is of opinion that direct stimulation of the longitudinal growth of intact roots by low auxin concentration has never been demonstrated as a regular reproducible phenomena. The reported

cases of auxin stimulation of root elongation has been interpreted as an adaptation of roots to higher auxin level; the stimulation seems to appear only after the disappearance of added auxin from the medium. But Moewus (1949) and Thimann (1952) reported that growth stimulation by auxin is more specific. Similarly other workers (Pohl 1951, 1952 and Pilet 1951, 1953) found striking stimulation of root elongation at low auxin concentration. Audus and Thresh (1953) also noticed 30 per cent increase in the root elongation after treatment with dil. IAA. Roberts and Street (1955), who isolated roots of rye which previously proved difficult to maintain in culture, noticed stimulation of growth in culture when applied with IAA of concentration $5 \cdot 7 \times 10^{-8}$ to $1 \cdot 8 \times 10^{-9}$ M.

The striking effect of the chemicals on the rooting of the rice plant appears to be of some practical significance. It is interesting to note that stimulation of root elongation occurs in rice. The number of lateral rootlets is considerably increased both in the higher and lower concentrations (Table 7). Åberg (1957) recorded no stimulation in root elongation by auxin in *Lepidium* which has got a tap root system while in the case of the fibrous root system of rice the rootlets originate from the base and it is evident that a large number of lateral rootlets come out from the region after auxin treatment and these are much longer than those in the control (Figs. 5 to 8). Root elongation was also noted in the treatment of seedlings for 6 days (Table 4). While in the experiments supplied with auxins for longer periods growth both in number and length is continued. Åberg's contention was that the stimulation occurs after the disappearance of auxin from the medium but in the present case the question of disappearance does not arise as supply was maintained regularly in the culture solution. Auxin analysis of the roots also show increasing auxin content and concentration after different treatments. Thus it appears that external supply of the regulators increases endogenous auxin level and this appears to be related with the increase in root growth. However, without further works it is not possible to offer adequate explanation for the mechanism of increased auxin concentration accelerating root growth by cell multiplication and elongation.

The increase in auxin level by the external application of the growth regulators is regularly seen in all the stages of the plants. In spite of the limited value of the use of root inhibition technique for the assay of auxin from plant materials it may be argued from the comparable results of the auxin levels of the organs from the treated and untreated plants that there occurs an increase in the endogenous auxin concentration after treatments with the growth regulators. The high auxin level after treatment with IAA is obviously related to the uptake of IAA by plant tissue. In comparison to IAA the auxin level is lower in NAA and MH treatments. The incredibly

high auxin level has been noted by Moewus (1949) in cress roots, in *Brassica* roots by Linser and Maschek (1953). But Audus and Thresh (1956) did not find any increase in pea roots with TIBA and 2–4, D. But auxin level was increased with MH. Åberg (1957) noted that no adequate data on the uptake of auxin in fibrous roots are available and experiments to show IAA accumulation in plants have failed. The results from the analysis of the rice plant show an accumulation in different organs after treatments with IAA (Tables 8 and 10 to 12). This accumulation very likely takes place in some form, from which it can be readily extracted in water. The question then arises what would be the effect of high internal concentration presumably of toxic level on the growth response of rice plants. Since root growth is not adversely affected, rather there occurs a stimulation, the toxicity of the endogenous level does not arise.

According to Åberg (1957) auxin occurs in two parts in plant tissues. He has suggested that the bulk of the auxin taken up by the tissue simply accumulates in the vacuoles where it remains in inactive form, while the active auxin part has ready access to the sites of growth. It is presumed that free auxin level in the sites of growth is below sub-optimal concentration as to bring about stimulation. The increased endogenous auxin level in rice plants seems to agree with the hypothesis advanced by Åberg. The high auxin level after treatment with NAA and MH needs some consideration in view of the conflicting results obtained by Audus and Thresh (1956), Pilet (1951, 1953) and Fransson (1958). With MH, Audus and Thresh (1956) showed slight increase in endogenous auxin while Pilet (1951, 1953) found no effect. But Fransson (1958) reported the action of anti-auxin, p-chloro-phenoxy-iso-butyric-acid (PCIB), raising the endogenous auxin of wheat roots. The increased auxin level after PCIB treatment has been interpreted by Fransson (1958) as a sort of competitive action between IAA and PCIB. As a result of competition at the site of action native auxin will be displaced from its natural active sites by the application of PCIB, consequently higher concentration of free auxin results. The same relation will possibly hold good for the effect of anti-auxin like MH on the endogenous auxin level of rice roots. It is to be noted that Fransson's (1958) experiment with PCIB showed stimulation of root growth which was also the case in rice roots of the experiments reported. The effect of NAA in increasing the auxin level may also be explained by noting the fact that NAA is a more congenial auxin for rooting than IAA (Audus 1953). Accordingly, the competition between IAA and NAA would result in freeing the IAA from the active sites of growth.

The whole problem of the relation of the growth regulators to endogenous auxin levels of rice roots appears to be complex, specially in view of the fact that rice plants are capable of forming a very massive, tufted, root system

from the base of the stem at a varying range of auxin concentration which would be toxic to many other plants.

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ORIGIN AND DEVELOPMENT OF FIBRES IN RAMIE (*BOEHMERIA NIVEA* GAUD.)

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ABSTRACT

The origin and cytohistological changes during the development of the phloem fibre cells of *Boehmeria nivea* Gaud. have been studied. The primary phloem fibres originate from the procambial cells below the shoot apex and in the elongating internode; they crush the proto-phloem elements by elongation and enlargement of their cells. They become several times longer than the neighbouring cells from which they are distinguishable by the belated appear-ance of vacuoles in the cytoplasm, and by multinucleate conditions resulting from successive nuclear divisions which are not usually followed by cytoplasmic divisions. Transverse septa have been found in a few young fibre cells.

Unlike the usual process of growth in which deposition of secondary wall layers begins after the cessation of the growth in extension of the primary fibre cell, in *Boehmeria nivea*, simultaneous growth resulting from the extension of the primary wall at the tip end and of deposition of the secondary wall layers at the morphological base of the fibre cell is the character-istic feature. In addition to the symplastic growth and the intrusive growth at the two ends of the fibre cells, the unusual length attained by them may be due to an independent mode of linear growth by which their apical ends with primary wall having abundant cytoplasm and a number of nuclei have an unlimited scope for growth by keeping pace with the tissues at the zone of active growth and elongation. The basal portion of the cell which remains at the level of origin shows restricted activity probably due to the surrounding mature tissue. This is evident from the spacing of the compartments formed by the ends of the successively laid down lamellae of the secondary wall near the two ends of the cell. Maturation of the fibre cell is acropetal, proceeding from its morphological base to the tip.

Secondary fibre cells, found in the mature stem, originate in the secondary phloem tissue and are scanty, uninucleate, much narrower and shorter than the primary fibres.

INTRODUCTION

Boehmeria nivea Gaud., belonging to the family Urticaceae, is an im-portant fibre yielding plant. The fibre cells are very long and often attain a length of 550 mm. or more; they are many times longer than the associated cells of the mature tissue and pass through several internodes in the mature stem. This unusual development in the length of the fibre cells is of parti-cular interest anatomically, and calls for a detailed study of its mode of origin and development. Aldaba (1927) has studied the mode of increase in length and secondary differentiation of the walls of the fibre cells and has

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observed that the process of elongation is gradual and extends over a number of months. Frey-Wyssling (1948) and Muhlethaler (1949) have studied the submicroscopic structure of the fibre cell wall and the intermicellar system by X-ray and electron microscopic analysis. Kundu and Rao (1957) have studied the morphogenesis at the shoot apex. So far as the authors are aware, the ontogeny of the fibre cell, its subsequent stages of maturation and cytological features during development have not yet been studied in detail. The present study reveals the mode of origin and cytohistological changes during the development of fibre cells in ramie.

MATERIAL AND METHODS

The materials have been collected from two strains of plants grown in the farm of the Jute Agricultural Research Institute at Nilganj, Barrackpore. The stem apices and the successive internodes below the apical bud have been fixed in Navashin's fluid and F.A.A. on the spot, dehydrated in grades of normal butyl alcohol and embedded in paraffin following the usual schedule. Longitudinal and transverse microtome sections have been cut 5 μ to 10 μ thick according to the stages required and slides stained in haematoxylin-safranin or safranin-fast green stain combinations. Stem apices and elongating internodes have been macerated in EDTA (Letham 1958) and mature shoots in 5 per cent KOH (Aldaba 1927).

Epidermal hairs in the immature portion of the stem are removed as far as practicable by scraping with fine dissecting needles; the pith and xylem portions are also peeled out carefully. Stem apices and narrow strips of bark of the young internodes are taken on slides and squashed in 10 per cent glycerine. Mature fibres are floated on water in a tray and mounted on ten inches long slides by drawing up slowly from the bottom of the tray to keep the fibres stretched on the slide and also to facilitate the marking of morphological polarity of the fibre cells. Where necessary the macerated materials are stained with aceto-orcein (2 per cent) or safranin (0·5 per cent) aqueous.

OBSERVATIONS

The shoot apex is concave and sunken below the leaf primordia (Kundu and Rao 1957). The procambium strands can be traced up to the base of the first leaf primordium, in the actively growing shoot apices developing in continuation with the older strands below (Fig. 5). That the longitudinal course of development of the procambial strands is acropetal, is clearly evident from the size and shape of the cells which become typically narrow, long and fusiform in the downward direction, while at the apex they are comparatively less differentiated than the surrounding meristematic cells (Figs. 17 and 18). Due to denser cytoplasm of these cells, the procambium

strand takes a differential staining and in transverse section it can be easily recognized from the surrounding parenchymatous cells of the pith and cortex (Figs. 1–3). The procambium strand becomes increasingly well defined due to repeated longitudinal divisions of the cells at various planes, thereby giving rise to a strand with random arrangement of the cells which are elongated in shape (Figs. 5 and 19).

Vascular development starts by the initiation of the protophloem elements at the outer periphery of the procambial strand usually 63 μ to 84 μ below the actively growing shoot apex (Figs. 3 and 4). The prosenchymatous cells surrounding the protophloem elements elongate simultaneously and become as long as the sieve elements (Sen and Kundu, unpub.) 100 μ to 200 μ below the shoot apex, the walls of the protophloem elements show gradual collapse though the surrounding cells, which ultimately form the fibres, are still full of cytoplasm and show active division of the nucleus. The protophloem elements are ultimately crushed by the expansion of the surrounding cells and are discernible for a time as thick and deeply stained spots between the developing fibre cells (Fig. 6). In cross-sections at this level, the young fibre cells are not quite recognizable from the neighbouring cells by their shape or cytoplasmic content except from their comparatively thick walls (Figs. 7 and 8).

In the elongating internodes (Fig. 9) these young fibre cells are fusiform in shape with attenuated ends and abundant cytoplasm. A number of vacuoles are observed near the oblong or spindle-shaped nucleus. The nucleus usually divides and two to four nuclei are formed in one fibre cell (Figs. 10, 11 and 12). In a few cases a transverse septum has been found to divide a fibre cell unequally into two compartments (Fig. 11).

The young fibre cells usually elongate at a faster rate than the surrounding tissues in the elongating internodes; thickening of their walls begins particularly at the basal end of the cells simultaneously with the elongation of the cells. At this stage abundant cytoplasm with more than one nucleus is found at the apical end of the young fibre cells (Figs. 13 and 13a) while the basal end of the cells shows a large vacuole with scanty cytoplasm lining the thickened cell wall. Granular materials are seen in the empty lumen of the cell. Subsequent elongation of the fibre cells is predominantly dependent on the activity of the immature apical portion. By virtue of its plastic primary wall, this portion elongates keeping pace with the growth of the surrounding cells. In macerated materials fibre cells have been found with their basal end at the level of secondary growth of the stem having thick secondary wall and empty lumen while the apical end is still at the level of the elongating internode having thin primary wall with some amount of cytoplasm and disorganizing spindle-shaped nuclei (Fig. 20).

In cross-section the young fibre cells are angular in outline like the

surrounding phloem-parenchyma cells. With maturity the cells become oval or roundish in outline having thick secondary wall and narrow lumen (Fig. 14). In some of the cultivated races of ramie, the secondary wall is comparatively thin and the lumen is wide (Fig. 15).

Macerated mature fibres are cylindrical, with attenuated end and many transverse markings (Figs. 20A, 20A_1, 20A_2). The tips of the cells are rounded and often found to be dovetailed with the neighbouring fibre cells (Figs. 20A and 20B). The thick secondary wall shows longitudinal fissures and long slit-type simple pits lying parallel to the long axis of the fibres (Fig. 21). The middle portion of the fibre is two to four times wider than of the end portions; the basal end is wider than the apical portion.

The secondary wall layers are deposited from the basal portion by means of thin cellulose layers on that portion of the fibre which is not actively growing. These layers are deposited gradually and as the apical end of the fibre is growing in length, the layers formed later are longer than the earlier formed ones. The open ends of these layers which are deposited one upon another form some sort of compartments within the fibres. The lumen appears to be highly uneven due to various degrees of contraction of the open end of these lamellae, which often partially or wholly block the lumen. Transverse septa observed in a few young fibre cells are never found in mature fibre cells. In some fibres, cytoplasm with degenerating nucleus at the apical end has often been found (Fig. 20A). Granular chromatic materials and often a few polyhedral clustered oxalate crystals have also been found in the empty lumen (Fig. 20A_2). In cross-sections the fibres are round or elliptical. The secondary walls often show infolding while the primary walls retain the oval or round shape. After swelling of the wall, usually 3 layers (S_1, S_2, S_3) are seen, of which the middle one is very thick as compared to the outer and inner layers (Figs. 16 and 21). The thick middle layer shows the presence of further lamellae under high power of microscope. These layers are made up of spiral striations. The pitch of these striations and their radial orientation in cross-sections have been studied by a number of workers and these works have been reviewed by Roelofsen (1959). Transverse folds are observed at places on the mature fibres (Fig. 20C); such folds are slightly oblique and form an angle with the longitudinal axis of the fibre. These markings usually take up intense stain in safranin or aceto-orcein.

The fibre length usually varies from 48 mm. to 290 mm. and in diameter from 0·05 to 0·1 mm. (Table I). The longest fibres are usually found in the median region of the mature stem where the internodes are also longest as compared to those of the basal portion of the stem.

Secondary bast fibres are seen in the mature stem. These are very few in number, scattered into strands of two to three fibre cells originating by the activity of the cambium in the secondary phloem tissue, and are always

uninucleate. These fibres are much narrower and very much shorter than the primary fibres with average length of 15·5 mm. and diameter of 0·025 mm. (Table I). The cambium cells, from which they originate, are usually 0·53 mm. long and 0·025 mm. broad.

TABLE I

Measurement of fibres (in mm.)

|	Length		Breadth		Lumen	
	Range	Average	Range	Average	Range	Average
Primary fibre ..	48-290	164·5	0·05-0·1	0·075	0·015-0·035	0·025
Secondary fibre ..	10-22	15·5	0·016-0·035	0·025	*0·030-0·085 0·008-0·018	*0·060 0·013

* From the wide lumen variety.

DISCUSSION

The extraxylary fibres in *Boehmeria nivea* have been found to originate from the prosenchymatous cells around the protophloem elements. These cells have a common origin from the procambial cells from which the protophloem elements have originated. As such, ontogenetically the extraxylary fibres can be designated as phloic or phloem fibres.

Elaboration and maturation of the fibre initials are not, as in case of the neighbouring parenchymatous cells, immediately associated with vacuolation of cytoplasm which, however, begins later. The nucleus divides successively and two or more nuclei are formed. Nuclear division is not usually followed by cytokinesis and this results in multinucleate fibre cells. Similar multinucleate cells have also been noted in Linum (Haberlandt 1914), *Nicotiana* and *Linum* (Esau 1938 and 1943) and Cannabis (Kundu 1942). Occasionally, transverse septa have been observed in a few young fibre cells, which are elongating by symplastic growth of the primary wall in the elongating internodes. Transverse septa have, however, not been found in any mature fibre cells, indicating that the cell wall, if formed after nuclear division, disintegrates during maturation and growth in length of the fibre cells which, therefore, retain their multinucleate nature.

In a fibre cell, maturation proceeds from its morphological base upwards. The basal portion of a cell shows quite advanced stage of development due to the presence of a large vacuole and a thin layer of cytoplasm lining the wall or loss of cytoplasm and thickening of wall due to secondary wall development while the apical portion, with comparatively thin wall, abundant cytoplasm with small vacuoles and a few nuclei, is still undergoing elongation
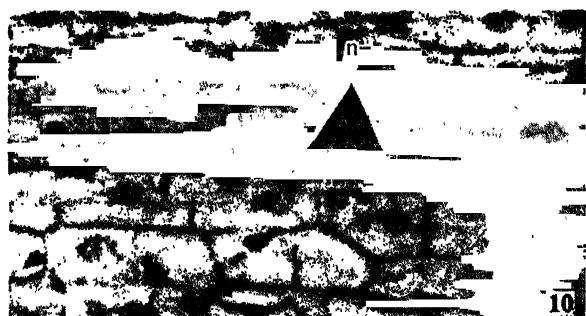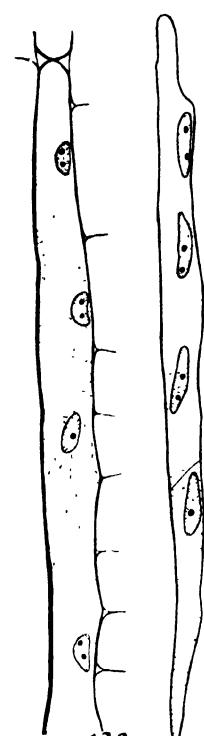
in the actively elongating internodes. This process of secondary thickening of wall from base to apex has also been seen by Anderson (1927) and Aldaba (1927) in *Boehmeria* and by Anderson (1927) and Tammes (1907) in *Linum*, and has been discussed by Esau (1953).

In phloem fibres, the deposition of secondary wall usually begins after primary wall approaches the end of its growth in extension. In *Boehmeria*, however, the sequence of events, i.e. cell elongation and secondary wall deposition, is simultaneous. This co-ordinated process of elaboration and development keeps pace in the elongating zone of the stem, particularly with reference to the apical end of the fibre cells. From longitudinal sections of stems of different ages, it appears that the young fibres grow symplastically along with the growth of the adjacent parenchyma cells. When the elongation of these parenchyma cells ceases further elongation of the fibre cells takes place by intrusive growth (Sinnott and Bloch 1939) of the two ends, indicated by the extremely attenuated ends dovetailing with the neighbouring fibre cells in the mature tissue. The elongation of the lower end lying within the mature tissues soon ceases, while the upper end continues to elongate. This method of elongation may be due to an independent mode of linear growth by which their apical ends with primary wall having abundant protoplasm and a number of nuclei have an unlimited scope of growth by keeping pace with the tissues at the zone of active growth and elongation. The prolonged elongation of the fibre cell extending through several internodes is an intricate process and results in a complicated mode of secondary wall development.

According to Aldaba (1927), the phenomenon of secondary wall formation in the fibre cells may be visualized ' as the telescoping of a series of successively formed open tubular, hyaline membranes within an elongating closed tubular membrane ' of the primary wall. Each ' tubular hyaline membrane ' is longer than the one immediately preceding. The ends of these successively formed layers partially interrupt the lumen forming ' compartments ', which are abundant near the end of the cells, the protoplasm, however, remaining in unbroken continuity. The acropetal mode of growth in length of the fibre cells is also evident from the distribution of the ' compartments ' by the successively deposited ' tubular hyaline membrane ' of the secondary wall. The basal portion has limited capacity for symplastic growth as it is located in the comparatively mature tissue and as such the ' compartments ' are closely situated. In contrast, the apical portion of the same cell, situated in the actively elongating zone, has capacity for growth and extension of its primary wall to keep pace with the newly maturing cells at that level. The apical portion thus offers a greater space in length for the ' tubular hyaline membrane ' of the secondary wall, thereby leading to a wider spacing of the ' compartments '.

Secondary phloem fibres arise from cambial derivatives in a region of
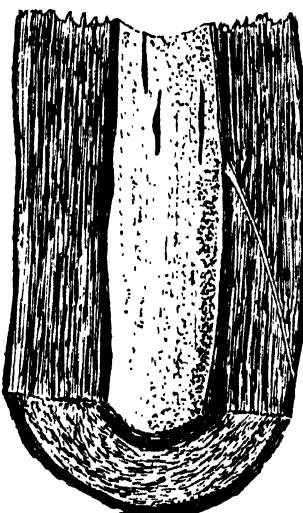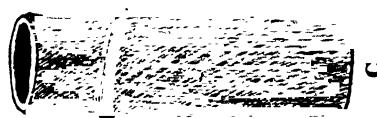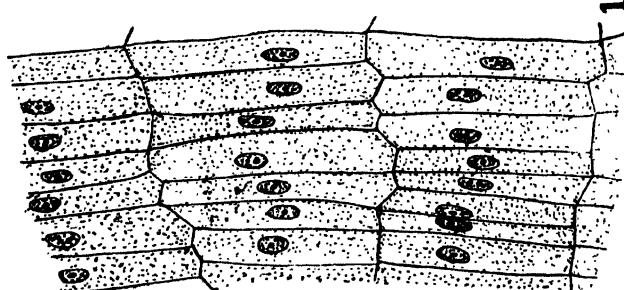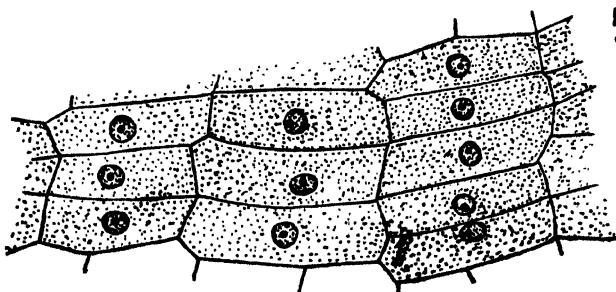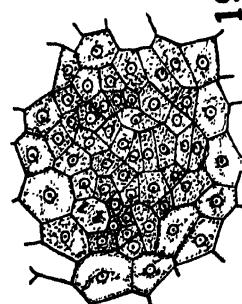
the stem that has ceased elongation. These fibres are much shorter than the primary fibres; they are, however, much longer than the cambial cells from which they are derived. The two ends of these fibres are narrower than the middle regions. The method of elongation of these fibre cells may be explained in the following way: After their development from the cambium initials, the differentiating fibre initials grow for a short while symplastically for the readjustment of the cells; thereafter, the two ends increase in length by intrusive growth.

In specially mounted cross-sections of the phloem's fibres Roelofsen (1959) has also found three layers in the secondary wall, as observed by us. The detailed microscopical study of the orientation of the spiral striation constituting these layers, by several workers, reveals the presence of considerable variation in the structure of the wall. The discrepancies, according to Roelofsen (*l.c.*), may be due to the fact that the S_1 layer (outermost layer) in ramie is very thin and in commercial fibres often entirely absent.

The transverse markings or 'slip planes' on the wall, according to Frey-Wyssling (1948), is due to dislocation and bending of the microfibrils. This also explains the greater penetration of the staining reagents in the comparatively wider intermicellar spaces which are not filled in by lignin as in the lignified fibre cells. The transverse markings are usually common in phloem fibres with little or no lignification of the wall.

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FIGURE INDEX

Plate XV

Figs. 1 to 4. *Boehmeria nivea* Gaud. Figs. 1 to 4. Serial transverse section of the shoot apex at the level of the apical meristem and 42 μ, 63 μ and 105 μ below the apical meristem respectively showing the tissue differentiation and elaboration of procambial strands ($\times$ 65). Fig. 5.

Median longitudinal section of the shoot apex showing the procambial strand in longitudinal course ($\times$140). Figs. 6 to 8. A portion of the vascular tissue in serial transverse section of the stem 210 μ, 350 μ and 500 μ below the apical meristem respectively, showing the development of the phloic fibres (Fig. 6$\times$250; Figs. 7 and 8$\times$125). Fig. 9. Transverse section of the young stem showing vascular strands capped by developing phloic fibres (dark stained) ($\times$80). a = apical meristem; pc = procambial strand; d = degenerated protophloem elements; f = fibre cells; pm = phloem and xm = xylem.

Plate XVI

Figs. 10 to 16. *Boehmeria nivea* Gaud. Fig. 10. Longitudinal section of stem showing a young fibre cell with two spindle-shaped nuclei ($\times$300). Fig. 11. Camera-lucida drawing of a young macerated fibre cell showing four nuclei and transverse wall ($\times$850). Fig. 12. Macerated young fibre cell with two nuclei ($\times$300). Figs. 13 and 13a. Photomicrograph and camera-lucida drawing respectively of the apical end of an elongating fibre cell showing vacuolated cytoplasm and four nuclei ($\times$240 and $\times$500). Figs. 14 and 15. Transverse section of stem of two cultivated varieties with narrow-lumened and wide-lumened fibre cells respectively ($\times$125). Fig. 16. Transverse section of the fibre cells showing the gross lamellation of the secondary wall ($\times$850). n = nucleus.

Text-figures

Figs. 17 to 21. *Boehmeria nivea* Gaud. Figs. 17 and 18. Longitudinal sections of procambial strand 35 μ and 55 μ below the shoot apex respectively ($\times$550). Fig. 19. Transverse section of the procambial strand 63 μ below the shoot apex ($\times$450). Figs. 20A, A_1, A_2 portions of the apical end of maturing fibre cell showing degenerated nuclei; granules, open ends of secondary wall lamellae forming compartments and clustered crystals; B = basal end of the same fibre showing dovetailing of the attenuated tip; C = lower-middle portion of the same fibre shown in surface view, one transverse fold is visible ($\times$350). Fig. 21. Semi-diagrammatic view of a portion of fibre cell cut longitudinally to show the extended and narrow simple pits and gross orientation of the secondary wall.

EMBRYOLOGY AND TAXONOMY OF THE SANTALALES—I

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Abstract

Comparative embryology of the Santalales indicates that the family Opiliaceae should be included as a tribe Opilieae under the Santalaceae, and the family Octoknemataceae should be merged with the Olacaceae. Moreover, the Olacaceae need not be raised to an ordinal rank. As compared to four tribes (Santalées, Osyridées, Comandrées and Thesiées) proposed by Van Tieghem (1896a), and three tribes (Anthoboleae, Osyrideae and Thesieae) by Engler and Diels (1936), in our opinion the family Santalaceae should comprise six tribes (Comandreae, Thesieae, Osyrideae, Santalaceae, Opilieae and Anthoboleae).

Further, the two subfamilies of the Loranthaceae, Loranthoideae and Viscoideae, should be upgraded to the rank of families and designated Loranthaceae and Viscaceae respectively.

Thus, the order Santalales should consist of two suborders, namely Santalineae and Loranthineae. The Santalineae should include four families (Olacaceae, Grubbiaceae, Santalaceae and Myzodendraceae) in contrast to six families (Olacaceae, Opiliaceae, Octoknemataceae, Grubbiaceae, Santalaceae and Myzodendraceae) proposed by Engler and Diels (1936). The suborder Loranthineae should include only two families, Loranthaceae and Viscaceae.

This revision is no doubt supported by embryological data, but for a conclusive classification we must await further researches on the morphology, anatomy, embryology and cytology of the uninvestigated genera of the Santalales.

Introduction

The embryology of the Santalales has attracted much attention for over a hundred years (*see* Griffith 1836a, b, 1843; Guignard 1885; Billings 1932, 1933; Rutishauser 1934, 1935, 1937; Schaeppi and Steindl 1937, 1942, 1945; Fagerlind 1947a, b, 1948, 1959; Singh 1952; Paliwal 1956). During the last ten years the following plants have been investigated in this department:

Santalineae—*Comandra umbellata* (Ram 1957); *Exocarpus cupressiformis, E. sparteus* and *E. strictus* (Ram 1959a); *Leptomeria acida* and *L. cunninghamii* (Ram 1959b); *Exocarpus bidwillii* (Bhatnagar 1959a); *Santalum album, S. freycinetianum* and *S. obtusifolium* (Bhatnagar 1959a, b); *Mida salicifolia* (Bhatnagar 1960); *Thesium alpinum* and *T. wightianum* (Bhatnagar and Agarwal 1960); and *Osyris wightiana* (Joshi 1960).

Loranthineae—*Helixanthera ligustrina* (Maheshwari and Johri 1950); *Macrosolen cochinchinensis* (Maheshwari and Singh 1952); *Scurrula cordifolia, S. ferruginea, S. parasitica* and *S. pulverulenta* (Agrawal 1953, 1954) ; *Lysiana exocarpi, Dendrophthoe neelgherrensis* and *D. falcata* (Narayana 1954, 1955, 1956, 1958a); *Nuytsia floribunda* (Narayana 1955, 1958b); *Amyema congener,*

A. miquelii, A. pendula and *A. preisii* (Dixit 1956, 1958*a*); *Lepeostegeres gemmiflorus* (Dixit 1958*b*); *Tolypanthus involucratus* and *T. lagenifer* (Dixit 1956, 1960); *Arceuthobium minutissimum* and *Korthalsella opuntia* (Correa 1958); *Atkinsonia ligustrina* (Garg 1958; *see also* Prakash 1960*b*); *Peraxilla tetrapetala* (Prakash 1960*a*); and *Barathranthus axanthus* and *Tapinostemma acaciae* (Garg 1959*a*, *b*).

At present work is in progress on some other members of the Santalaceae, Olacaceae and Loranthaceae: *Quinchamalium chilense*, *Olax wightiana, O. scandens, Strombosia ceylanica, Phrygilanthus tetrandrus, P. verticillatus* and *Psittacanthus cuneifolius*.

Before considering the embryological data, the families assigned to the order Santalales by various taxonomists may be mentioned:

Bentham and Hooker (1883): Loranthaceae, Santalaceae and Balanophoraceae.

Engler and Prantl (1889): Loranthaceae, Myzodendraceae, Santalaceae, Grubbiaceae, Olacaceae and Balanophoraceae.

Van Tieghem (1896*a*): Schoepfiacées, Arionacées, Sarcophytacées, Santalacées, Myzodendracées, Opiliacées and Anthobolacées.

Schellenberg (1932): Olacaceae, Grubbiaceae, Santalaceae, Myzodendraceae and Loranthaceae.

Wettstein (1935): Santalaceae, Grubbiaceae, Opiliaceae, Octoknemataceae, Olacaceae, Myzodendraceae, Loranthaceae, Balanophoraceae and Cyanomoriaceae.

Hutchinson (1959): Loranthaceae, Santalaceae, Grubbiaceae, Myzodendraceae and Balanophoraceae.

Engler and Diels (1936) divided the order Santalales into two suborders: the ,Santalineae which included six families—Olacaceae, Opiliaceae, Octoknemataceae, Grubbiaceae, Santalaceae and Myzodendraceae—and Loranthineae with a single family Loranthaceae.

Bentham and Hooker (1862–1867) raised the family Olacaceae to the ordinal rank, Olacales, and on the basis of separate petals placed it under the Polypetalae. He included three families in this order: Olacineae, Illicineae and Cyrilleae.

Van Tieghem (1896*a*) accepted the order Olacales but included only three families: Harmandiacées, Aptandracées and Olacacées. Hutchinson (1959) also retains the order Olacales but assigns six families: Olacaceae, Opiliaceae, Octoknemataceae, Aptandraceae, Dipentodontaceae and Medusandraceae.

Generally the three families Olacaceae, Opiliaceae and Octoknemataceae are included in the order Olacales (*see* Reed 1955). Van Tieghem (1896*a*) retained Olacaceae under the Olacales while Opiliaceae was transferred to the Santalales.

Comparative embryology and relationships of the Santalineae, Loranthineae, and inter-relationships of these suborders have been discussed in the following pages.

COMPARATIVE EMBRYOLOGY AND RELATIONSHIPS OF THE SANTALINEAE

Of the six families in the suborder Santalineae, embryology of only the Santalaceae, Olacaceae and Opiliaceae is somewhat better known while studies on Octoknemataceae, Grubbiaceae and Myzodendraceae are far from satisfactory.

SANTALACEAE—The pollen grains may be spherical (Fig. 1) or oblong (Fig. 2). The ovary is unilocular or 2- to 5-chambered at the base and unilocular above as in *Leptomeria* (Ram 1959b) and *Osyris* (Joshi 1960). The placental column may be extremely reduced as in *Exocarpus* (Figs. 10, 11), rather short as in *Osyris* (Fig. 8) (Guignard 1885; Joshi 1960), long and straight as in *Santalum* (Fig. 9) (Paliwal 1956; Bhatnagar 1959a), or long and twisted as in *Thesium* (Fig. 7) (Schulle 1933: Paliwal 1956) and *Comandra* (Figs. 5, 6) (Ram 1957). In *Exocarpus* (Fagerlind 1959) the placenta does not show any ovular differentiation† but in other members there are one to five ovules. They may be pendulous as in *Santalum* (Paliwal 1956), hemianatropous as in *Mida* (Bhatnagar 1960), or anatropous as in *Comandra* (Fig. 6) and *Osyris*. The ovules may not show any distinction into the integument and nucellus as in *Santalum* and *Mida*, or may be poorly differentiated, the nucellus being represented by only one or two epidermal cells as in *Leptomeria* and *Osyris*, or the ovules may have a distinct integument as in *Comandra* and *Thesium*. The embryo sac is of the Polygonum type (Figs. 15, 17, 18) and the micropylar end may (Fig. 9) or may not (Figs. 6, 7, 16) grow beyond the ovule or placenta (Fig. 11). Usually a caecum develops from the chalazal end and the antipodal cells are left *in situ* (Fig. 17). In *Comandra*, instead of a chalazal caecum, a lateral caecum arises near the egg apparatus (Fig. 15). Prominent haustorial processes develop from the embryo sac in *Exocarpus menziesii* (Fagerlind 1959). The endosperm may be Cellular as in *Thesium* (Fig. 22) (Schulle 1933; Paliwal 1956), *Osyris* (Fig. 23) and *Comandra* (Fig. 24); or Helobial as in *Santalum* (Paliwal 1956; Bhatnagar 1959a) and *Mida* (Bhatnagar 1960). A chalazal endosperm haustorium is common to several genera and it usually remains uninucleate (Figs. 22, 23, 25) except in *Exocarpus* (Ram 1959a; Bhatnagar 1959a) where it becomes multicellular (Fig. 26). Secondary endosperm haustoria develop in *Comandra* (Fig. 24) and *Mida*. The zygote divides transversely in *Exocarpus* (Fig. 29) and *Leptomeria* (Fig. 30), and vertically in *Comandra* (Fig. 31).

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† A normal ovule comprises the integument, the nucellus and the embryo sac. In *Exocarpus*, however, the ovule is much reduced, and the so-called erect and undifferentiated ovule is really the central placenta itself (*see also* Fagerlind 1959).
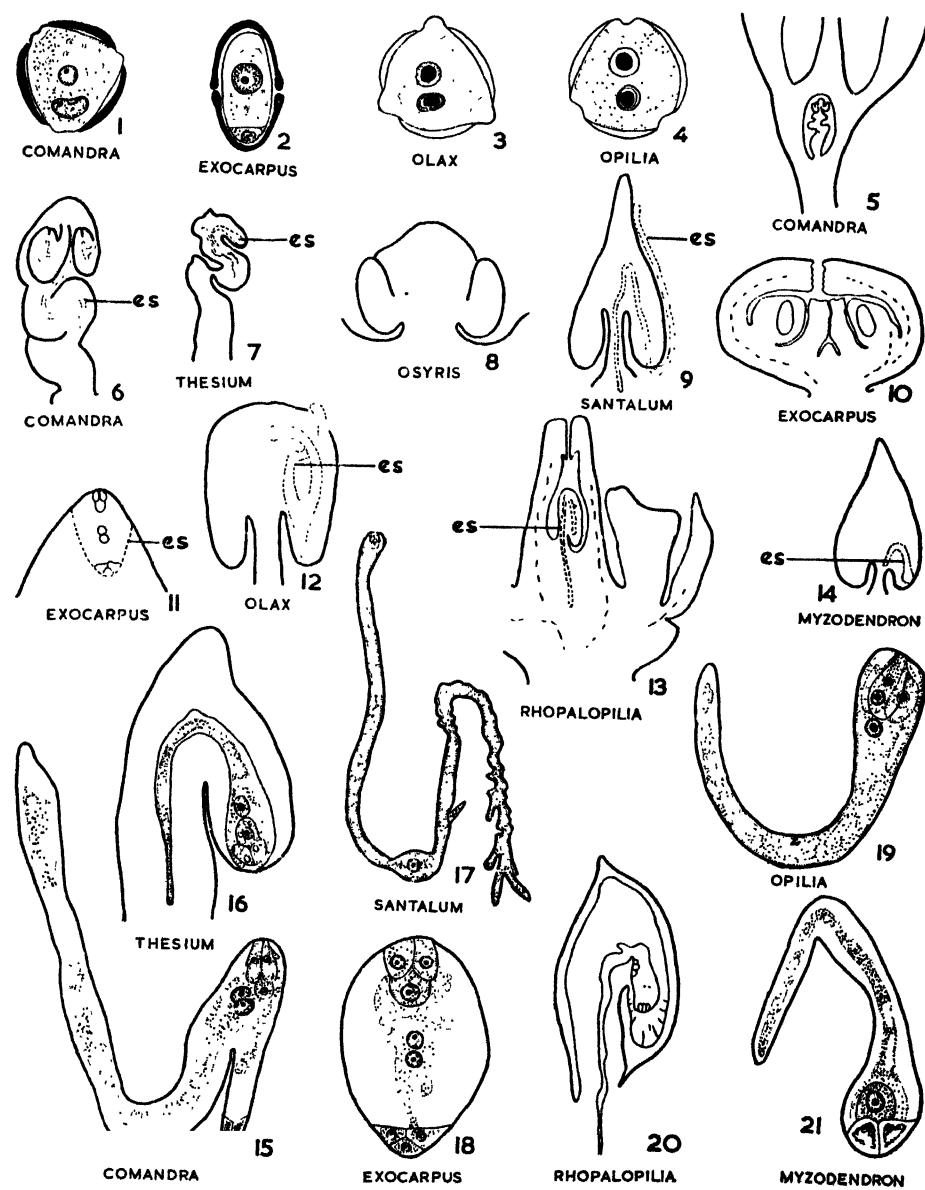
FIGS. 1–21.

On the basis of structure of the gynoecium, Engler and Prantl (1889) divided the Santalaceae into three tribes: Anthoboleae, Osyrideae and Thesieae. This arrangement was accepted by Pilger (1935), Engler and Diels (1936), Smith and Smith (1942) and Paliwal (1956).

Depending upon whether the placentae and ovules were straight or curved, Van Tieghem (1896a) divided the Santalaceae into four tribes: Santalées, Osyridées, Thesiées and Comandrées. He assigned *Arjona* and *Quinchamalium* to a separate family Arionacées because (a) the disc is epigynous instead of calycinal (calyx) as in the Santalaceae, (b) in *Arjona* the hairs at the base of the stamens arise from the epidermal cells of the perianth and not from the hypodermis as in the Santalaceae, and (c) the ovary is unilocular in the upper region and plurilocular with one ovule in each locule in the basal portion. He concluded that these differences justified the removal of *Arjona* and *Quinchamalium* from the Santalaceae and their grouping into the family Arionacées.

As far as the nature of the disc is concerned, the studies of Smith and Smith (1942) on the floral anatomy of the Santalaceae indicate that the presence of an epigynous disc is not a distinctive feature. As regards the origin of hairs, Van Tieghem's observations are incorrect since the hairs arise from the epidermal and not the hypodermal cells of the perianth. This is also true of *Arjona*. Lastly, in some genera like *Choretrum* and *Leptomeria* the ovary is plurilocular at the base and unilocular above. Thus, in this respect also *Arjona* and *Quinchamalium* are in no way different from the rest of the Santalaceae.

Moreover, *Arjona* and *Quinchamalium* show other resemblances with the Santalaceae, e.g. (a) central placental column bearing pendulous ovules which lack a distinction into the nucellus and integument, and (b) seeds without a testa (*see* Van Tieghem 1896a). Therefore, there is no justification for assigning *Arjona* and *Quinchamalium* to a new family Arionacées, and they should be retained in the Santalaceae (*see also* Pilger 1935).

Miers (1878) placed *Arjona*, *Myoschilos* and *Quinchamalium* in the Olacaceae. In these plants, according to him, the flowers are dichlamydeous (calyx is always free from the corolla) and the calyx is supported by a free calycle. Of these three genera, the embryology of only *Quinchamalium* has so far been investigated (Agarwal 1960). In spite of some differences like the formation of conspicuous synergid and antipodal haustoria, there are many similarities with the Santalaceae, e.g. the ovary is plurilocular below and unilocular above, the free central placenta bears three ovules which do not show any distinction into the nucellus and integument, the micropylar chamber produces the endosperm proper and development of endosperm haustoria from the chalazal chamber.

Therefore, until the other two genera are also investigated, it would be

22 THESIUM

23 OSYRIS

24 COMANDRA

25 SANTALUM

27 GRUBBIA

26 EXOCARPUS

32 COMANDRA

31 COMANDRA

30 LEPTOMERIA

29 EXOCARPUS

28 MYZODENDRON
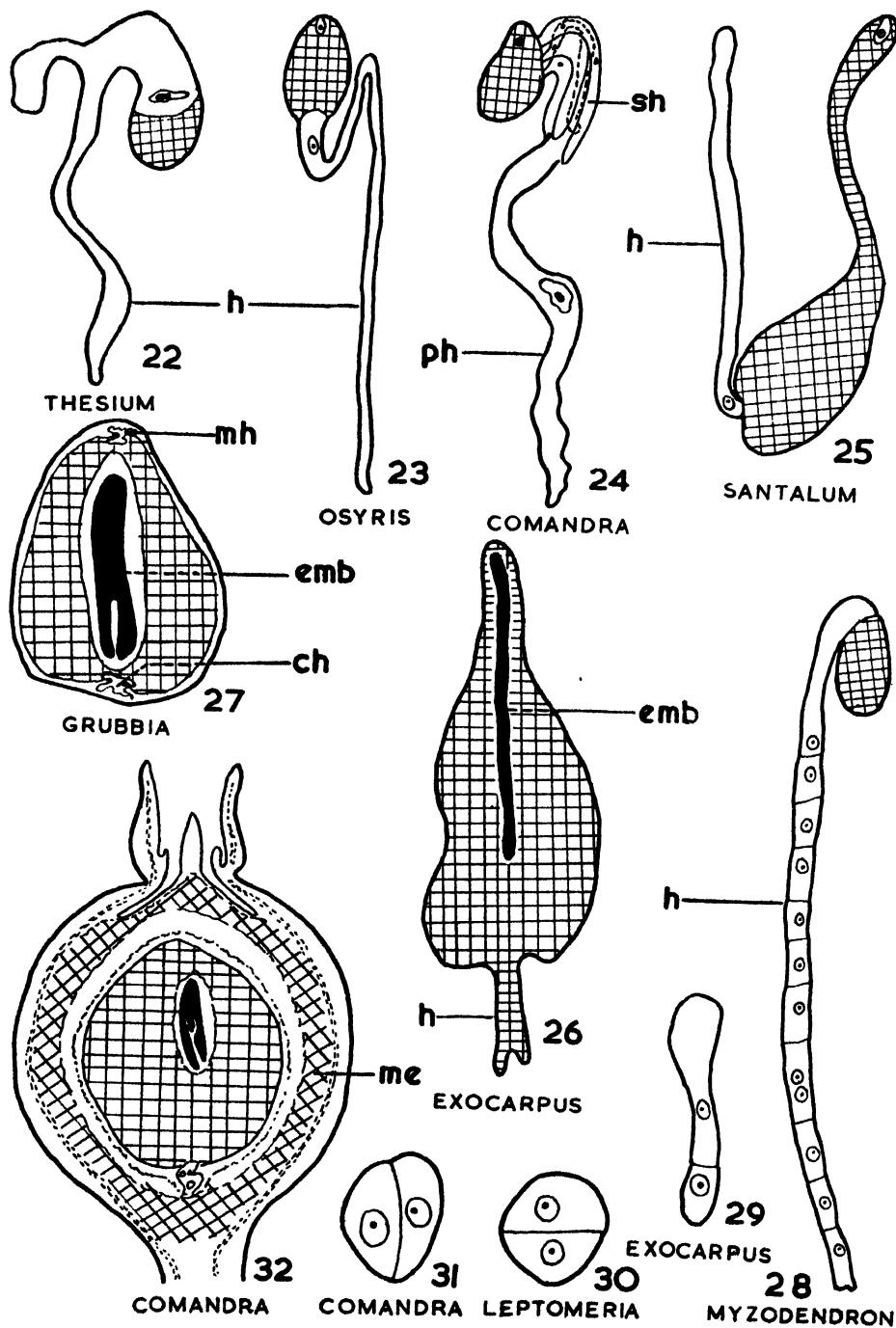
FIGS. 22–32.

premature to remove *Arjona, Myoschilos* and *Quinchamalium* from the Santalaceae.

The systematic position of *Exocarpus* has been quite uncertain. Van Tieghem (1896a) assigned *Anthobolus* and *Exocarpus* to a separate family, the Anthobolacées. Gagnepain and Boureau (1947) suggested a drastic change and transferred *Exocarpus* to the gymnosperms, while Lam (1948a, b) grouped it under the Protangiospermae along with *Salix* and *Casuarina*. Bailey (1949), Swamy (1949) and Eames (1951) have commented on the views of Gagnepain and Boureau, and Lam. They have emphasized that *Exocarpus* is an undoubted angiosperm. Suessenguth (1954) pointed out that the embryological study may be helpful in determining its systematic position.

The embryology of *Exocarpus* has recently been investigated by Ram (1959a) and Fagerlind (1959). The anthers are dithecous and pollen is shed at the 2-celled stage. There is a much reduced placenta (Fagerlind 1959). The hypodermal archesporial cell functions directly as the megaspore mother cell and the embryo sac conforms to the Polygonum type. In *E. menzeisii* the embryo sac produces extensive haustorial processes. The endosperm is Cellular with a well-developed chalazal haustorium. The zygote divides transversely and the mature embryo is typically dicotyledonous. These findings clearly show that *Exocarpus* possesses characteristic santalaceous features and there is not the slightest indication of any relationship with the gymnosperms. Swamy (1949) and Eames (1951) have also stated that *Exocarpus* is a reduced but phylogenetically advanced member of the Santalaceae (*see also* Maheshwari 1958). Therefore, Van Tieghem's assignment to an independent family Anthobolacées and Lam's temporary erection of the order Exocarpales may be rejected.

On the basis of embryology and other evidences, in our opinion, the following six tribes should constitute the family Santalaceae:

COMANDREAE—Ovary semi-inferior with a twisted placental column bearing two to four anatropous, unitegmic ovules; formation of a lateral caecum near the tip of the embryo sac; endosperm Cellular; presence of secondary endosperm haustoria; and longitudinal division of the zygote: *Comandra*.

THESIEAE—Ovary inferior with a twisted placental column bearing three pendulous, straight, unitegmic ovules; embryo sac remains confined to the ovule; endosperm Cellular; and transverse division of the zygote: *Thesium*.

OSYRIDEAE—Ovary partly or completely inferior with a short placental column bearing three anatropous, undifferentiated ovules; embryo sac shows slight protrusion owing to the dissolution of ovular epidermis; endosperm Cellular; and transverse division of the zygote: *Osyris*.

SANTALEAE—Ovary semi-inferior with a long, straight, placental column bearing three pendulous and undifferentiated ovules; embryo sac extends

33 SCURRULA

34 ATKINSONIA

35 PHORADENDRON

36 ARCEUTHOBIUM

37a

38a

39a

40a

46a

37b PERAXILLA

38b MACROSOLEN

39b HELICANTHES

40b HELIXANTHERA

46b

HELIXANTHERA
TUPEIA
DENDROPHTHOE
NUYTSIA
HELICANTHES
TOLYPANTHUS
LYSIANA
LEPEOSTEGERES
ATKINSONIA
MACROSOLEN

45

ant
cae

44 LEPEOSTEGERES

41 AMYEMA

42a

42b AMYEMA

43 MACROSOLEN

46c

46d

KORTHALSELLA 46e
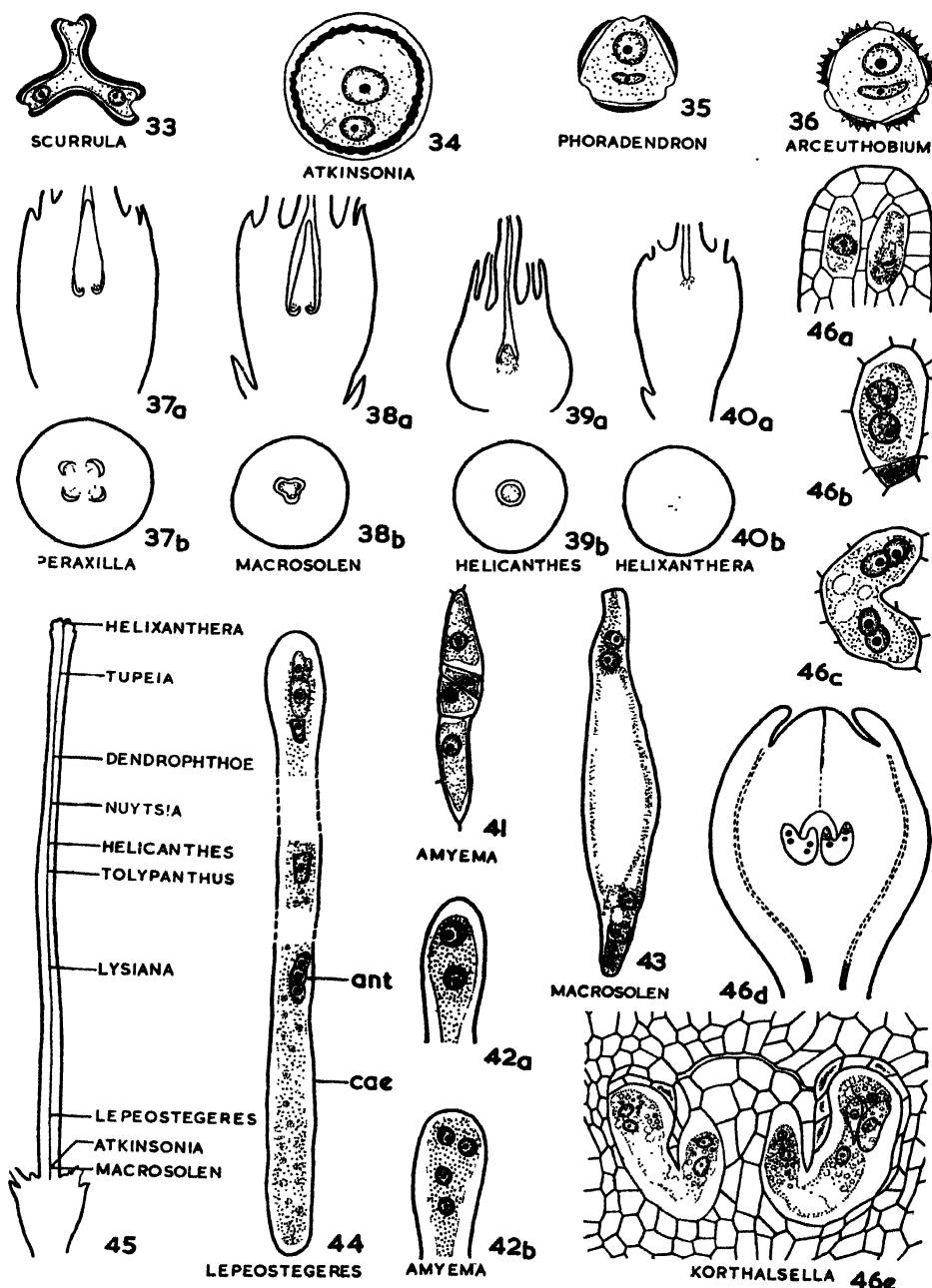
FIGS. 33–46e.

beyond the ovule; endosperm Helobial or Cellular; and transverse division of the zygote: *Leptomeria, Mida* and *Santalum.*

OPILIEAE—Ovary superior with a single pendulous ovule or ovule absent; embryo sac remains confined to the ovule; endosperm Cellular; and presence of secondary endosperm haustoria: *Cansjera* and *Opilia.*

ANTHOBOLEAE—Ovary semi-inferior with a much reduced central placenta, ovules absent; embryo sac remains confined to the placenta; endosperm Cellular; and transverse division of the zygote: *Exocarpus.*

OLACACEAE—Van Tieghem (1896a), Reed (1955) and Hutchinson (1959) recognized an independent order, Olacales. To determine whether this is justified, the more important morphological and embryological features of the families Santalaceae and Olacaceae are compared in the following table (*for literature see* Agarwal 1960; Bhatnagar 1959a, 1960; Fagerlind 1947a, 1948, 1959; Joshi 1960; Paliwal 1956; Ram 1957, 1959a, b; Reed 1955; Shamanna 1954; Smith and Smith 1942):

| CHARACTER	SANTALACEAE	OLACACEAE
HABIT	Root parasites.	Autotrophic or root parasites.
FLOWER	Unisexual or bisexual; monochlamydeous; disc present.	Unisexual or bisexual; mono- or dichlamydeous; disc present.
ANTHER TAPETUM	Glandular; 1, 2 or multinucleate cells.	Glandular; 2- or 4-nucleate cells.
POLLEN	Tricolpate; 2- or 3-celled.	Tricolpate; 2- or 3-celled (Fig. 3).
OVARY	Inferior or semi-inferior; unilocular or 2- to 5-chambered at base and unilocular above.	Superior, inferior or semi-inferior; unilocular, sometimes chambered at base and unilocular above.
OVULE	One to five; pendulous, hemianatropous or anatropous; borne on a central placenta; differentiation into nucellus and integument lacking (*Santalum*). or unitegmic (*Thesium*).	One to five; anatropous; borne on a central placenta; differentiation into nucellus and integument lacking (*Olax imbricata*), or unitegmic (*Olax wightiana, Strombosia*), or bitegmic (*Coula, Ximenia*).
EMBRYO SAC	Polygonum type; tip of embryo sac may or may not extend beyond the ovule; chalazal caecum develops.	Polygonum or Allium type; micropylar end may (Fig. 12) or may not extend beyond the ovule; chalazal caecum develops.
ENDOSPERM	Cellular or Helobial; chalazal endosperm haustorium unicellular or multicellular; secondary haustoria may also develop.	Cellular; chalazal endosperm haustorium unicellular.
EMBRYO	Zygote divides transversely or longitudinally; suspensor absent or present.	Zygote divides transversely; suspensor present.
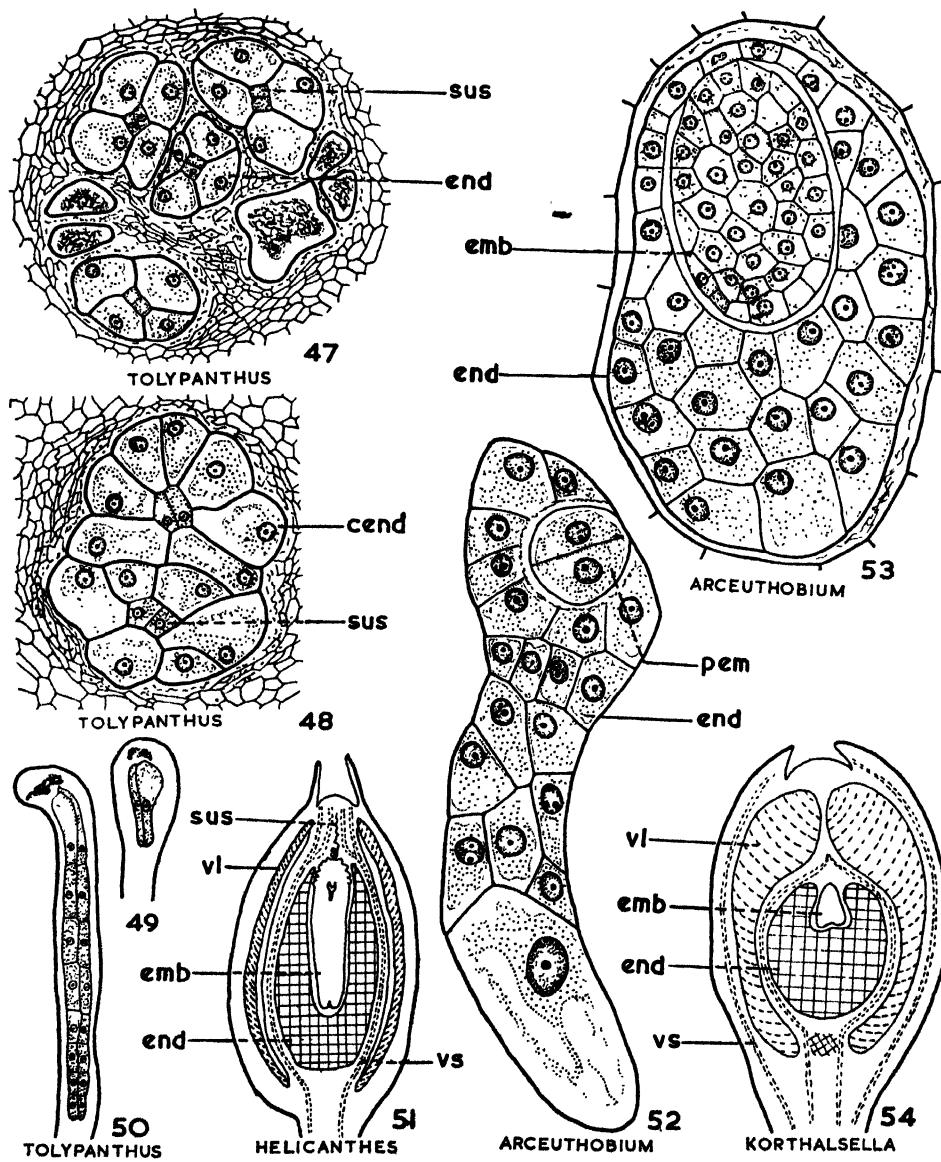
Figs. 47-54.

Thus, it is obvious that there are strong similarities between the Santalaceae and Olacaceae. The latter have two important features of contrast too : (1) presence of calyx, and (2) bitegmic ovules in some cases. We may now consider some other evidences also.

As early as 1851, Miers drew attention to the fact that the floral morphology of the Olacaceae and Santalaceae have several common features. In the olacaceous genera the flowers are distinctly dichlamydeous. In the Santalaceae the outer indentation in the flowers of *Choretrum* and *Mida* represents a reduced calyx, which during the course of evolution has become obliterated in other genera of this family. The epigynous gland so highly developed in some members of the Olacaceae is an equally constant feature of the Santalaceae.

A comparison of the placental-ovular-complex of the Olacaceae and the Santalaceae led Fagerlind (1947a, 1948) to conclude that the two families are closely related to each other. Smith and Smith (1942) provided further evidence of a close alliance between these families on the basis of floral anatomy, and they pointed out that the Santalaceae have probably been derived from the Olacaceae.

Therefore, there does not seem to be sufficient justification to raise the family Olacaceae to the rank of an order as suggested by Van Tieghem (1896a), Reed (1955) and Hutchinson (1959).

OPILIACEAE—According to Fagerlind (1948) the placental-ovular-complex and the embryo sac of the Opiliaceae remind one of *Thesium*, and he suggested that this family should be included as a tribe Opilieae under the Santalaceae. The Opiliaceae show a solitary undifferentiated ovule which may be pendulous or erect, e.g. *Rhopalopilia* (Figs. 13, 20). In *Cansjera* the placenta and the ovule are further reduced, and in *Agonandra* the ovary shows a reduced placenta while the ovule does not differentiate 'at all. The latter genus, therefore, is possibly the nearest approach to *Exocarpus*. Also, in the Opiliaceae (Shamanna 1955; Swamy 1960) the pollen grains are spherical (Fig. 4) and the embryo sac is of the Polygonum type (Fig. 19) resembling that of *Thesium* (Fig. 16) (Shamanna 1955). In *Cansjera* (Swamy 1960) the endosperm is Cellular and both primary and secondary haustoria are formed—a feature common to some members of the Santalaceae, e.g. *Comandra* and *Mida*. Thus, the inclusion of Opiliaceae as the tribe Opilieae (Fagerlind 1948) under the Santalaceae appears to be in order.

OCTOKNEMATACEAE—Only two genera, *Octoknema* and *Okoubaka*, have been assigned to this family (*see* Reed 1955). Fagerlind (1948) and Stauffer (1957) stated that, as far as the structure of the wood, pollen grains, ovary, placenta and development of the embryo sac are concerned, *Octoknema* resembles the members of the Olacaceae. Stauffer, therefore, concluded that the family

Octoknemataceae can no more be held valid and its merger with the Olacaceae would be appropriate.

The genus *Okoubaka* also shows some features common to the Santalaceae, e.g. presence of hairs at the base of the perianth lobes and unilocular ovary with a free central placental column bearing three anatropous and undifferentiated ovules. On the basis of these similarities, Stauffer (1957) suggested the transfer of *Okoubaka* to the Santalaceae. The structure of the wood of this plant also suggests affinities with the Santalaceae (Normand 1944). Occasionally, the trees growing in the neighbourhood of *Okoubaka* die, indicating the parasitic nature like that of the Santalaceae.

GRUBBIACEAE—Endlicher (1840) and De Candolle (1857) established Grubbiaceae as a distinct family, and kept it near the Santalaceae. Bentham and Hooker (1883) did not recognize the family status and reduced it to a tribe Grubbieae under the Santalaceae.

Prior to Van Tieghem's (1896a) observations the Grubbiaceae were considered to possess an inferior, unilocular ovary and a free central placenta bearing at its tip two orthotropous, naked ovules. According to him the ovary is bilocular and the delicate partition easily disorganizes giving the false impression of a unilocular condition. The placentation is axile and in each locule there is an anatropous, unitegmic ovule, and the integument transforms into a seed coat. Thus, the structure of the flower and fruit of *Grubbia* not only differs from that of the Santalaceae, but also from other families included in the Santalineae. Therefore, Van Tieghem (1897) pointed out that the correct place of Grubbiaceae was near the Bruniaceae, order Hamamelidales.

Fagerlind (1947b) confirmed Van Tieghem's views and stated that *Grubbia* is related to the Bicornes (Ericales). The thick integument shows a long micropyle and due to degeneration of the nucellus the tip of the embryo sac comes to lie in the micropyle. Both micropylar and chalazal endosperm haustoria are formed (Fig. 27). The epidermal layer of the endosperm is covered with a thick cuticle and the reserve food in the endosperm and embryo is proteinaceous. The embryo occupies the entire length of the seed.

Most of the embryological features referred to by Fagerlind are also common to the Santalaceae. Further, both uni- and bitegmic ovules are found in the allied family Olacaceae and here the micropyle is also quite conspicuous. In most genera of the Santalaceae the endosperm is proteinaceous and shows a cutinized epidermis (*see* Schulle 1933; Ram 1957, 1959a, b). Moreover, in *Santalum* also the mature embryo occupies the entire length of the seed (Pilger 1935; Bhatnagar 1959a).

On the basis of vegetative anatomy, Metcalfe and Chalk (1950) stated that *Grubbia* differs from the Santalaceae and should be assigned to a separate family, the Grubbiaceae. According to Erdtman (1952), the pollen grains of

14B

Grubbiaceae also differ from those of the Santalaceae and resemble those of the Ericaceae.

There is no doubt that *Grubbia* has some distinctive features of its own which warrant the erection of an independent family, the Grubbiaceae. Concerning its placement, the many similarities, especially the central placenta, unitegmic ovules, cutinized epidermis of the endosperm, and the embryo occupying the entire length of the seed, speak of a relationship with the Santalaceae and, therefore, there is every justification to include it in the Santalineae.

MYZODENDRACEAE—Bentham and Hooker (1883) assigned the genus *Myzodendron* to the tribe Osyrideae under the Santalaceae. Rendle (1952) also confirmed that " The genus *Myzodendron* may be regarded as a much reduced ally of the Santalaceae ". The common features between *Myzodendron* and Santalaceae are : receptacular ovary, three pendulous and undifferentiated ovules borne on a free central placenta (Fig. 14), Polygonum type of ⌐- or ⌐-shaped embryo sac (Fig. 21) (Johnson 1889 ; Skottsberg 1913, 1935), and Cellular endosperm with a chalazal haustorium. At first the chalazal haustorium becomes divided into a row of cells (Fig. 28) but later, due to dissolution of the walls, the haustorium appears multinucleate.

Some of the important dissimilarities between the Santalaceae and *Myzodendron* are : naked male flowers, monothecous anthers; forate, spheroidal and spinuliferous pollen grains; female flowers with three hairy, stiff and persistent bristles alternating with the stigma; and a rudimentary testa. A satisfactory taxonomic assignment must await further work on this interesting genus but even at present the erection of the family Myzodendraceae appears to be necessary.

COMPARATIVE EMBRYOLOGY AND RELATIONSHIPS OF THE LORANTHINEAE

As pointed earlier, the suborder Loranthineae comprises a single family, the Loranthaceae, with two subfamilies: Loranthoideae and Viscoideae. Danser (1929, 1933*a*, *b*) divided the Loranthoideae and Viscoideae into three tribes each :

LORANTHOIDEAE—Elytrantheae, Lorantheae and Nuytsieae.

VISCOIDEAE—Phoradendreae, Arceuthobieae and Visceae.

The distinctive embryological features of these tribes are given below :

LORANTHOIDEAE

ELYTRANTHEAE

(*a*) Pollen grains triradiate.

(*b*) Tricarpellary gynoecium ; 3- or 4-lobed placenta (mamelon) with the same number of chambers in the ovary (Figs. 37*a*, *b*) : *Lysiana* (Narayana 1958*a*), *Peraxilla* (Prakash 1960*a*) ; or the placenta may be lobed but it

remains free from the ovary wall: *Macrosolen* (Figs. 38*a*, *b*) (Maheshwari and Singh 1952).

(*c*) One to four achesporial cells develop in each lobe of the placenta: *Elytranthe* (Schaeppi and Steindl 1942; Agrawal 1953), *Macrosolen* (Maheshwari and Singh 1952); or the archesporium may be massive: *Lysiana* (Narayana 1958*a*).

(*d*) Three to five embryo sacs develop in the same ovary and they may extend only up to the base of the style: *Macrosolen* (Fig. 45) (Maheshwari and Singh 1952); or slightly above the base of the style: *Lepeostegeres* (Fig. 45) (Dixit 1958*b*); or up to half the length of the style: *Lysiana* (Fig. 45) (Narayana 1958*a*).

(*e*) Endosperm Cellular, lobed composite structure which extends around the hypostase (collenchymatous tube).

(*f*) Dicotyledonous embryo with free cotyledons (except in *Lysiana* where they are slightly fused along the margin).

(*g*) Fruit is a ' pseudoberry '.

LORANTHEAE

(*a*) Pollen grains triradiate, spherical in *Tupeia* (Smart 1952).

(*b*) The structure of the placenta is very variable. It may be well developed and free from the ovary wall: *Helicanthes* (Figs. 39*a*, *b*) (Johri, Agrawal and Garg 1957); or

A rudimentary placenta may be formed due to pushing up of the base of the ovarian cavity during elongation of the sporogenous tissue: *Amyema* (Dixit 1958*a*), *Tolypanthus* (Dixit 1960); or

The placenta may be rudimentary or absent: *Dendrophthoe* (Narayana 1956; Singh 1952), *Scurrula* (Schaeppi and Steindl 1942; Agrawal 1953); or

The placenta may be altogether absent; *Helixanthera* (Figs. 40*a*, *b*) (Maheshwari and Johri 1950), *Tupeia* (Smart 1952), *Tapinanthus* (Dixit 1956), *Barathranthus* (Garg 1959*b*).

(*c*) Massive archesporium.

(*d*) Tips of 4 to 12 embryo sacs ascending up to different heights in the style and stigma (Fig. 45).

(*e*) Endosperm Cellular, unlobed composite structure which does not extend around the hypostase.

(*f*) Embryo with fused cotyledons except in the region of plumule.

(*g*) Fruit is a ' pseudoberry '.

NUYTSIEAE

(*a*) Pollen grains triradiate in *Nuytsia* (Narayana 1958*b*) and spherical in *Atkinsonia* (Fig. 34) (Prakash 1960*b*).

(b) Tricarpellary gynoecium, 3-chambered ovary and 3-lobed placenta: *Nuytsia* (Narayana 1958b).

(c) Massive archesporium: *Nuytsia* (Narayana 1958b).

(d) Four or five embryo sacs reaching up to the base of the style in *Atkinsonia* (Fig. 45) (Prakash 1960b) and 8 to 12 embryo sacs up to two-thirds the length of the style in *Nuytsia* (Fig. 45) (Narayana 1958b).

(e) Lateral caecum at upper end of the embryo sac in some genera: *Nuytsia* (Narayana 1958b), *Atkinsonia* (Prakash 1960b).

(f) Endosperm Cellular, unlobed composite structure in *Nuytsia* (Narayana 1958b), or lobed as in *Atkinsonia* (Prakash 1960b).

(g) Embryo with free cotyledons.

(h) Fruit sub-drupaceous: *Nuytsia* (Narayana 1958b), or ' pseudodrupe ': *Atkinsonia* (Prakash 1960b).

The tribe Nuytsieae resembles Elytrantheae as far as characters (b) and (g) are concerned, and the tribe Lorantheae in characters (c) and (f).

VISCOIDEAE

PHORADENDREAE

(a) Pollen grains spherical, 2-celled: *Phoradendron* (Fig. 35) (Billings 1932); *Korthalsella* (Correa 1958).

(b) A central placenta develops at the base of the ovary, ovules absent: *Phoradendron* (Billings 1933), *Korthalsella* (Fig. 46d) (Rutishauser 1935, 1937; Correa 1958).

(c) Embryo sac U-shaped: *Phoradendron* (Billings 1933), *Korthalsella* (Figs. 46c–46e) (Rutishauser 1935; Correa 1958).

(d) Endosperm Cellular, derived from a single embryo sac: *Korthalsella* (Correa 1958), *Phoradendron* (Billings 1933).

(e) Zygote divides vertically, suspensor absent: *Korthalsella dacrydii* (Rutishauser 1935), *K. opuntia* (Correa 1958).

ARCEUTHOBIEAE

(a) In the anther the archesporium forms a continuous ring around a sterile columella.

(b) Pollen grains spherical, 2-celled: *Arceuthobium* (Fig. 36) (Correa 1958).

(c) A central placenta develops at the base of the ovary, ovules absent.

(d) Embryo sac straight and oriented obliquely within the placenta.

(e) Endosperm Cellular, derived from a single embryo sac: *Arceuthobium* (Figs. 52, 53) (Correa 1958).

(f) Zygote divides vertically, suspensor absent: *Arceuthobium minutissimum* (Fig. 52) (Correa 1958).

VISCEAE

(a) Anthers polysporangiate (Schaeppi and Steindl 1945).

(b) Placenta absent, archesporium differentiates from the cells at the base of the ovary.

(c) Embryo sac straight.

(d) Endosperm Cellular, derived from a single embryo sac.

(e) Zygote divides transversely; short suspensor present: *Viscum album* (Schaeppi and Steindl 1945).

As early as 1851, Miers treated the Loranthaceae and Viscaceae as separate families but most other taxonomists (Engler 1888-1889; Danser 1929, 1933a, b) treat them as subfamilies. In recent years considerable embryological data have accumulated and Maheshwari (1954, 1958), and Maheshwari, Johri and Dixit (1957), who have discussed their relationships, suggest that they should be raised to the rank of families.

Whether this is justified or not, would be clearly brought out from a comparison of their morphological and embryological characters:

CHARACTER	LORANTHOIDEAE	VISCOIDEAE
HABIT	Stem parasites; some root parasites, e.g. *Nuytsia* and *Atkinsonia*.	Stem parasites.
LEAF	Leathery.	Leathery; sometimes absent.
FLOWER	Unisexual or bisexual; calyculus invariably present (vascular supply develops only in *Nuytsia* and *Atkinsonia*); ovary may contain a lobed placenta which may be free or fused with the inner wall of ovary in between the lobes, or placenta may be unlobed and conical, or it may be altogether absent; hypostase saucer-shaped as in *Nuytsia* (Narayana 1958b) or tubular as in *Lysiana* (Narayana 1958a), *Lepeostegeres* (Dixit 1958b) and some other plants.	Unisexual; calyculus reported only in staminate flowers of *Viscum articulatum* and *V. orientale* (Schaeppi and Steindl 1945); a central placenta develops at the base of ovary in most species, placenta reduced in *Viscum*; ovules do not differentiate at all.
POLLEN GRAIN	Triradiate (Fig. 33), except *Tupeia* (Smart 1952) and *Atkinsonia* (Fig. 34) (Prakash 1960b) where they are spherical; 2-celled.	Spherical (Figs. 35, 36); 2-celled.
EMBRYO SAC	Polygonum type (Figs. 41–44); several embryo sacs develop concurrently in the same ovary; their tips reach up to the base of the style, or up to different heights in the stylo and stigma (Fig. 45); the lower end produces a caecum leaving the antipodal cells *in situ* (Fig. 44).	Allium type (Figs. 46a, b); embryo sac is usually straight but it is obliquely oriented in *Arceuthobium*; in *Korthalsella* at the 4-nucleate stage the 'lower' end bends (Fig. 46c), extends beyond the papilla and proceeds upwards towards the carpellary tissue (Figs. 46d, e).

CHARACTER	LORANTHOIDEAE	VISCOIDEAE
ENDOSPERM	Cellular; composite structure formed by the fusion of different endosperms developing in the same ovary (Figs. 47, 48).	Cellular; develops individually in each embryo sac (Figs. 52, 53) and do not fuse.
EMBRYO	Division of zygote always vertical (Figs. 49, 50); suspensor present (Fig. 51).	Division of zygote transverse except in *Korthalsella* (Rutishauser 1935; Correa 1958) and *Arceuthobium* (Fig. 52) (Correa 1958) where it is vertical; suspensor usually absent (Fig. 53).
PERICARP	Distinguishable into three zones : outermost leathery coat is followed by the viscid and parenchymatous zones; viscid layer situated outside the vascular supply to corolla (Fig. 51).	Distinguishable into three zones: outer fleshy coat is followed by the viscid and parenchymatous zones; viscid layer situated internal to the vascular supply to perianth (Fig. 54); in *Korthalsella* (Fig. 54) viscid layer is surrounded by the vascular tissue on either side.

Thus, the Loranthoideae and Viscoideae differ in their floral structure, mode of development of the embryo sac, endosperm, embryo and pericarp and, therefore, they should be raised to the family rank and designated Loranthaceae and Viscaceae respectively. Further, in view of their common habit, absence of ovules and presence of viscid layer in the pericarp their retention in the suborder Loranthineae is quite appropriate.

INTER-RELATIONSHIPS OF THE SANTALINEAE AND LORANTHINEAE

Whereas the order Santalales generally includes two suborders, Loranthineae and Santalineae, Van Tieghem (1896b) erected the orders Loranthales and Viscales and placed them in the subclass Inovulées near the Innucellées comprising two orders, Olacales and Santalales.

We have already shown that the Loranthoideae and Viscoideae should be raised to the family rank but whether they should be upgraded to independent orders (*see* Van Tieghem 1896b) or retained under the Santalales (*see* Engler and Diels 1936) deserves careful attention. To decide this issue the morphology and embryology of the Loranthineae and Santalineae may be compared :

CHARACTER	SANTALINEAE	LORANTHINEAE
HABIT	Autotrophic (Olacaceae) or root parasites (Santalaceae).	Usually stem parasites; exceptionally (*Nuytsia* and *Atkinsonia*) root parasites.
LEAF	Alternate; sometimes reduced to scales (*Exocarpus*).	Usually opposite, often leathery and persistent.

CHARACTER	SANTALINEAE	LORANTHINEAE
FLOWER	Unisexual or bisexual; usually monochlamydeous, dichlamydeous in Olacaceae; calyculus present in *Choretrum, Mida* and some other members of Santalaceae.	Unisexual or bisexual; calyculus invariably present in Loranthaceae and rarely in Viscaceae.
POLLEN GRAIN	Spherical or oblong; 2- or 3-celled (Figs. 1–4).	Triradiate or spherical; 2-celled (Figs. 33–36).
OVARY	Superior, semi-inferior or inferior; one-celled or 2 to 5-chambered at base and unilocular above.	Inferior; one-celled, often 3- or 4-chambered, e.g. *Lepeostegeres, Lysiana, Nuytsia* and *Peraxilla*.
OVULE	Distinct ovules present on a central placenta (Figs. 5–9, 12–14) and may be undifferentiated into nucellus and integument or uni- or bitegmic; ovules absent in *Exocarpus* (Santalaceae) and *Agonandra* (Opiliaceae).	Ovules (in the usual sense) absent, the Loranthaceae show a placenta (mamelon) which may be lobed and attached to ovary wall between the lobes (Figs. 37a–40b); the Viscaceae also show a placenta (Figs. 46d, e).
EMBRYO SAC	Polygonum type (Figs. 15–21); the embryo sacs may remain limited to the ovule or the tip may grow beyond the ovule towards the stylar canal; chalazal caecum is formed; plants like *Comandra* show a micropylar caecum.	Polygonum type in the Loranthaceae (Figs. 41–44) and Allium type in the Viscaceae (Figs. 46a–e); the tips of the embryo sacs grow only up to the base of the style in some genera and in others up to different heights of the style and stigma (Fig. 45); a chalazal caecum is formed; some plants like *Nuytsia* and *Atkinsonia* also show a micropylar caecum.
ENDOSPERM	Cellular (Figs. 22–24, 26–28, 32) or Helobial; chalazal haustorium present.	Cellular, composite endosperm in the Loranthaceae (Figs. 47, 48, 51), chalazal haustoria develop in *Tapinostemma* (Garg 1959a, b); endosperm derived from a single embryo sac in the Viscaceae (Figs. 52–54).
EMBRYO	Division of zygote usually transverse (Figs. 29, 30); longitudinal in *Comandra* (Fig. 31).	Division of zygote usually transverse in the Viscaceae and invariably vertical in the Loranthaceae (Fig. 49).
TESTA	Even though the ovules may be uni- or bitegmic, the integuments are consumed by the embryo sac and endosperm and, therefore, there is no testa.	Since even the ovules are absent, there is no testa.
PERICARP	Pericarp consists of the parenchymatous epicarp, stony mesocarp, and parenchymatous endocarp (Fig. 32); endocarp is consumed by the endosperm.	Pericarp consists of the outer leathery coat followed by the viscid layer and parenchymatous zone traversed by vascular strands (Figs. 51, 54).

Briefly then, both the Santalineae and Loranthineae show (a) a poorly or well-developed calyculus, (b) spherical pollen grains (Santalineae and Viscaceae, and *Tupeia* and *Atkinsonia* of Loranthaceae), (c) gradual reduction in the structure of the placental-ovular-complex in the Santalineae and placenta (mamelon) in the Loranthineae, (d) extra-ovular prolongation of embryo sac in some cases in the Santalineae and extension of tips of embryo sacs to different heights in the style and stigma of the Loranthineae, (e) formation of a caecum from the lower end of the embryo sac and sometimes from the tip (*Comandra* of Santalaceae, and *Nuytsia* and *Atkinsonia* of Loranthaceae), (f) formation of endosperm haustoria (Santalineae, and in *Tapinostemma* of Loranthaceae). and (g) vertical division of the zygote (*Comandra* of Santalaceae, Loranthaceae, and some members of Viscaceae).

The dissimilarities between the two suborders are : in the Santalineae the leaves are usually alternate, there are distinct ovules, tips of embryo sacs do not extend into the style, Cellular or Helobial endosperm, usually transverse division of the zygote, and absence of a viscid layer in the pericarp. Similarly, in the Loranthineae leaves are opposite and leathery, ovules are absent, embryo sacs grow to different heights in the style and stigma, composite endosperm in the Loranthaceae, vertical division of the zygote, fused cotyledons in some members, and presence of viscid layer in the pericarp.

Thus, embryological data support the division of the order Santalales into the suborders Loranthineae and Santalineae, and there seems to be no valid reason to erect two independent orders Loranthales and Viscales as had been done by Van Tieghem (1896b).

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EXPLANATION OF FIGURES

Figs. 1–21. Pollen, placental column and embryo sacs of the Santalineae (*es*—embryo sac). Figs. 1–4. Pollen grains. Fig. 1. *Comandra umbellata* (*After* Ram 1957). Fig. 2. *Exocarpus sparteus* (*After* Ram 1959a). Fig. 3. *Olax wightiana* (*After* Shamanna 1954). Fig. 4. *Opilia amentacea* (*After* Shamanna 1955). Figs. 5–14. Placental column, the outline of embryo sacs is shown with dotted lines. Figs. 5, 6. *Comandra umbellata* (*After* Ram 1957). Fig. 7. *Thesium montanum* (*After* Schulle 1933). Fig. 8. *Osyris alba* (*After* Schaeppi and Steindl 1937). Fig. 9. *Santalum album* (*After* Paliwal 1956). Figs. 10, 11. *Exocarpus sparteus* (*After* Ram 1959a). Fig. 12. *Olax wightiana* (*After* Shamanna 1954). Fig. 13. *Rhopalopilia umbellata* (*After* Fagerlind 1948). Fig. 14. *Myzodendron punctulatum* (*After* Skottsberg 1913). Figs. 15–21. Embryo sacs. Fig. 15. *Comandra umbellata* (*After* Ram 1957). Fig. 16. *Thesium montanum* (*After* Schulle 1933). Fig. 17. *Santalum album* (*After* Paliwal 1956). Fig. 18. *Exocarpus*

* Not seen in original.

sparteus (*After* Ram 1959*a*). Fig. 19. *Opilia amentacea* (*After* Shamanna 1955). Fig. 20. *Rhopalopilia umbellata* (*After* Fagerlind 1948). Fig. 21. *Myzodendron punctulatum* (*After* Skottsberg 1913).

FIGS. 22–32. Endosperm, embryo and fruit of the Santalineae (*ch*—chalazal endosperm haustorium, *emb*—embryo, *h*—haustorium, *me* —mesocarp, *mh* -micropylar endosperm haustorium, *ph*—primary haustorium, *sh*—secondary haustoria). Figs. 22–28. Endosperm. The cross-hatched region represents the cellular endosperm. Fig. 22. *Thesium montanum* (*After* Schullo 1933). Fig. 23. *Osyris wightiana* (*After* Joshi 1960). Fig. 24. *Comandra umbellata*; note the presence of secondary endosperm haustoria (*After* Ram 1957). Fig. 25. *Santalum album* (*After* Bhatnagar 1959*a*). Fig. 26. *Exocarpus bidwillii*; note the multicellular endosperm haustorium (*After* Bhatnagar 1959*a*). Fig. 27. *Grubbia tomentosa*; note the micropylar as well as the chalazal endosperm haustoria (*After* Fagerlind 1947*b*). Fig. 28. *Myzodendron punctulatum*, the endosperm haustorium is multicellular (*After* Johnson 1889). Figs. 29–31. Proembryo. Fig. 29. *Exocarpus sparteus*; division of the zygote is transverse (*After* Ram 1959*a*). Fig. 30. *Leptomeria cunninghamii* (*After* Ram 1959*b*). Fig. 31. *Comandra umbellata*; division of the zygote is vertical (*After* Ram 1957). Fig. 32. L.s. fruit of *Comandra umbellata* (*After* Ram 1957).

FIGS. 33–46*e*. Pollen, placenta and embryo sacs of the Loranthineae (*ant*—antipodal cells, *cae* -caecum). Figs. 33–36. Pollen grains. Fig. 33. *Scurrula pulverulenta* (*After* Agrawal 1953). Fig. 34. *Atkinsonia ligustrina* (*After* Prakash 1960*b*). Fig. 35. *Phoradendron flavescens* (*After* Billings 1932). Fig. 36. *Arceuthobium minutissimum* (*After* Correa 1958). Figs. 37*a*, *b*–40*a*, *b*. L.s. and t.s. of ovaries showing types of placenta in the Loranthaceae, the dotted regions indicate the sporogenous tissue. Figs. 37*a*, *b*. *Peraxilla tetrapetala* (*After* Prakash 1960*a*). Figs. 38*a*, *b*. *Macrosolen cochinchinensis* (*After* Maheshwari and Singh 1952). Figs. 39*a*, *b*. *Helicanthes elastica* (*After* Johri, Agrawal and Garg 1957). Figs. 40*a*, *b*. *Helixanthera ligustrina* (*After* Maheshwari and Johri 1950). Figs. 41–46*e*. Megasporogenesis and female gametophyte. Fig. 41. Linear tetrad of *Amyema miquelii* (*After* Dixit 1958*a*). Figs. 42*a*, *b*. Tips of 4- and 8-nucleate embryo sacs of *Amyema miquelii* (*After* Dixit 1958*a*). Fig. 43. Six-nucleate embryo sac of *Macrosolen cochinchinensis* (*After* Maheshwari and Singh 1952). Fig. 44. Eight-nucleate embryo sac of *Lepeostegeres gemmiflorus*; note the chalazal caecum (*After* Dixit 1958*b*). Fig. 45. L.s. carpel showing extension of embryo sacs in the style; the names indicate the level of the tips of embryo sacs in respective genera. Fig. 46*a*. *Korthalsella opuntia*; l.s. placenta showing two megaspore mother cells (*After* Rutishauser 1937). Figs. 46*b*, *c*. *K. dacrydii*; 2- and 4-nucleate embryo sacs (*After* Rutishauser 1935). Figs. 46*d*, *e*. *K. opuntia* (*After* Correa 1958). Fig. 46*d*. L.s. pistillate flower showing position of embryo sacs. Fig. 46*e*. Embryo sacs enlarged from previous figure.

FIGS. 47–54. Endosperm, embryo and fruits of the Loranthineae (*cend*—composite endosperm, *emb*—embryo, *end*—endosperm, *pem*—proembryo, *sus*—suspensor, *vl*--viscid layer, *vs*—vascular supply). Figs. 47–50. *Tolypanthus involucratus* (*After* Dixit 1960). Figs. 47, 48. T.s. portion of ovaries showing formation of composite endosperm. Figs. 49, 50. Two- and 16-celled proembryos; division of the zygote is longitudinal. Fig. 51. L.s. fruit of *Helicanthes elastica* (*After* Johri, Agrawal and Garg 1957). Figs. 52, 53. *Arceuthobium minutissimum* (*After* Correa 1958). Fig. 52. Two-celled proembryo and endosperm. Fig. 53. Globular proembryo with advanced stage of endosperm. Fig. 54. L.s. fruit of *Korthalsella opuntia* (*After* Correa 1958).

CLAN DEMOGRAPHY OF AN ABOR VILLAGE

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ABSTRACT

From the data presented, it is concluded that the Abor prefer to marry more within the village than outside it. This, limited by the small size of the mating groups (clans) and the number of the two sexes at the reproductive period of 15–49 years (male 174: female 169), appears to suggest high inbreeding among the Abor. At the same time the frequencies of un-married males (12 per cent) and females (18 per cent) are noteworthy features for an aboriginal population of the type of the Abor. Premarital licence is not restricted in the Abor society. According to Sarma, girls pregnant through dormitory alliances are not always married by the actual father of the child and such children accompany the mother to the new husband. This indicates the presence of recognized illegitimacy among the Abor, and that the dormitories of the lower regions, which include the village of Aieng, are also visited by the non-Abor men. How far inbreeding is responsible for the abnormal characters, like deaf-mutism, mental dis-orders, goitre, etc., is difficult to assess from the present data, but that such people find it almost impossible to get a mate is obvious from their unmarried status.

INTRODUCTION

The present work gives a detailed analysis of the demographic data collected during the author's visit to the Abor Hills in the North-East Frontier Agency in 1949-50 as a member of an anthropological party of the Depart-ment of Anthropology, Government of India. He was, however, sent for the purpose of carrying out ABO blood groups investigations. These data have already been published by Bhattacharjee (1958) who also carried out some work among the Abor during 1948-49.

The first village visited was Aieng, about 6 miles from Pasighat on the right bank of the Dihang river. The party stayed at this village from 9th to 27th December, 1949. During this period only 61 Abors could be blood-grouped (Table 2) out of a total population of 734 souls. Many a day was spent without work and it was during these leisure hours that a demographic study of the clans and of the whole village was planned.

Further it was apparent in course of the first few days' reconnoitring of the village that there were a large number of abnormal men and women. They are as follows:

There were two unmarried deaf-mutes, one of each sex, of about 30 years of age. In the Abor language a deaf-mute is known as *mide* and differ-entiated from an idiot or mentally defective by the term *rube*. The two deaf-mutes were the last born children of normal parents, being born at about the 30th year of their mother's age. The female deaf-mute happened to be

both an idiot and a deaf-mute and belonged to the Legaw clan. The other belonged to the Perme clan.

There appeared to be a large number of unmarrieds of both the sexes as well. Forty-three unmarried males between the ages of 20 and 50 years and 65 unmarried females between the ages of 14 and 30 years were counted. Their numbers in each group are as follows:—

TABLE 1

Number and age of unmarried men and women at Aieng

Age	Male	Age	Female
20	9	14	4
21	2	15	4
22	16	16	9
23	4	17	8
25	3	18	12
26	1	19	2
28	3	20	8
30	2	22	9
40	2	23	2
50	1	24	1
		25	1
	43	26	3
		30	2
			65

There were 12 goitrous men and women, whose details are given below:

Males with goitre

Clan	Age	Remarks
Perme	12	Unmarried, first child of normal parents, their ages are not known.
Legaw	50	Married, having a very big goitre filling the region of the throat completely, had five issues.
Perme	70	Fairly large goitre, married, had a son about 42 years old.

Females with goitre

Clan	Age	Remarks
Tayeng	15	Unmarried.
Irang	18	,,
Megu	26	,,
Perme	30	Married with two sons, first born two years old.
Perme	32	Married with two sons and two daughters.
Legaw	38	Married, barren.

Clan	Age	Remarks
Tayeng	55	Married with two sons and one daughter.
?	65*	Married with an unmarried son about 40 years old.
?	65*	Married with a son and a daughter, both married and having children.

It was not possible to classify the mental defectives and the Abor classification of *rube*, which appeared to be well recognized in the Abor society, was applied. Of the seven (four males and three females) mentally defectives, only two were married. Their details are as follows:

Clan	Age	Remarks
Tayeng	28	Unmarried, said to be of melancholic nature and also of violent temper sometimes.
,,	30	Unmarried, known as a *rube*, had also an unmarried father's younger brother, about 40 years old.
Perme	50	Unmarried, also a *rube*.
,,	52	Married, also recognized as a *rube*, had a married son and an unmarried daughter of 22 years; six children died young.

All the above four instances belong to males. Of the three females all were unmarried. One was a girl of 14 years of the Tayeng clan and appeared

TABLE 2

*Blood groups from Aieng**

Clans:	Borang		Irang		Legaw		Megu		Osik		Perme		Tayeng		Total
Blood group	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	
O	—	—	—	—	—	—	—	—	—	—	·	3	—	2	5
A	1	—	—	—	2	2	—	1	—	—	1	2	4	3	16
B	1	4	—	1	2	2	1	2	1	—	2	3	5	3	27
AB	—	—	—	—	1	—	1	1	—	—	3	1	3	3	13
	2	4	—	1	5	4	2	4	1	—	6	9	12	11	
	6		1		9		6		1		15		23		61

* Married women have been counted as belonging to her father's clan.

to be a Mongolian idiot. She was the last but one child of her parents and born at about 26 years of age of her mother. The other two women were

* Both married in Perme clan.

about 30 years old, of which one was a deaf-mute as already mentioned. The other belonged to the Tayeng clan and happened to be the second child of her parents. Her mental defect was not of a serious nature. Three of her younger sisters were married and gave birth to children.

All these prompted a study of the mating conditions of the Abor at this village. Due to lack of time and other difficulties towards the end of our stay, a proper study based on kinship relations could not be made as it required the records of earlier generations of each family. We had to confine ourselves within the existing members of a family. It may be noted here that the exchange of brothers and sisters is much in vogue at Aieng, as is also the case among other Abor divisions (Sarma 1960).

The Data

Aieng village* was situated on either side of a perennial stream, which supplied the water requirements of the village. The stream ran east to west and the huts were located on the north and the south. The southern area was a flat low hill on the slopes of which huts were raised. There were 111 huts at this area. The northern area, on the other side of the stream, was a plain land and comprised 20 huts.

The demographic data were collected by visiting each hut after dusk, when the Abor returned home from the field and had their dinner. A few huts required more than one visit at a convenient time suited to both of us, for completing details. The work was actually started on the 14th December, 1949, after about a week's association with the village. It was found during the collection of pedigrees that Abor matings were controlled through a number of clans and subclans, and their knowledge appeared to be a primary necessity.

The writer had also the opportunity of visiting three other Abor villages and in each village some data were collected but none was so thorough as that of Aieng. After Aieng, the next village visited was Mimesipo, which comprised 85 huts. From Mimesipo the party came to the village of Mebo on the 3rd January, 1950, and stayed till 18th January, reaching Pasighat the same day. Mebo was the largest of the four villages. According to local information there were 170 huts. Some data were also collected therefrom.

Clans and subclans

Abor clans are exogamous. Some of them are divided into subclans which are also exogamous. It was found that the numbers of clans are not uniformly distributed in each village.

The Abor of Aieng, Mebo, Dapui and Mimesipo mainly belong to the Padam division of the tribe, which is supposed to be endogamous in nature.

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* The topography of Aieng has been changed during the earthquake of 1952.

Both at Mebo and Mimesipo, Pasi families belonging to another endogamous division of the tribe were met with. It was held by the senior members of our party that Padam and Pasi are two different endogamous divisions of the Abor and this probably led Bhattacharjee to separate the blood group data accordingly. Enquiries at Mebo and Mimesipo, however, revealed that Padam × Pasi intermarriages were in vogue—its rarity was not noticed because of the small number of Pasi families. There were six Pasi families at Mimesipo, while at Mebo eight genealogies of Pasi × Padam intermarriages were recorded. From the inadequate data available to the present writer, it is seen that blood samples of 11 individuals out of such intermarriages were grouped. How they have been classified by Bhattacharjee is not intelligible from his paper.

The different clans met with in the different villages are given below. It may here be pointed out that the village of Aieng was thoroughly investigated—the other three villages could not be studied in all details. The Abor clans and subclans are given in Table 3.

TABLE 3

Abor clan names

Sl. No.	Clan	Subclans	Sections of subclans
		Village : Aieng	
1	Borang	(a) Litung	—
		(b) Lingyong	—
		(c) Luei	(i) Sitang
			(ii) Sipang
2	Irang	(a) Rinbang	—
		(b) Rinning	—
3	Legaw	(a) Rapok	—
		(b) Lagrang	—
		(c) Kopak	—
		(d) Lakku	—
4	Megu	(a) Domi	—
		(b) Nurang	—
		(c) Komji	—
		(d) Pebung	—
5	Modi	—	—
6	Osik	—	—
7	Perme	(a) Mekop	—
		(b) Mebang	—
		(c) Mei	—
8	Tayeng	(a) Subkom	—
		(b) Tonkir	—
		(c) Tonmuk	—
		(d) Kibing	—
		(e) Kirang	—
		(f) Dasum	—

TABLE 3—*contd.*

Sl. No.	Clan	Subclans	Sections of subclans
		Village : Mebo	
1	Borang	(a) Litung	—
		(b) Lithin	—
		(c) Lischi	—
		(d) Lingyong	—
		(e) Libol	—
2	Irang	(a) Bimul	—
		(b) Birang	—
		(c) Rinning	—
		(d) Rinsar	—
		(e) Numi	—
		(f) Peyang	—
		(g) Apum	—
3	Legaw	(a) Lagrang	(i) Kobing
			(ii) Kotem
			(iii) Kobang
			(iv) Rapok
		(b) Lakku	—
		(c) Kopak	--
4	Lingiligaw	(a) Jonang	—
		(b) Lomsar	---
		(e) Lombo	—
		(c) Lompir	—
		(d) Ayom	—
		(f) Lonjuk	—
5	Megu	(a) Nubung	--
		(b) Nurang	—
		(c) Komji	—
		(d) Domi	—
6	Parthin	(a) Pulling	--
		(b) Pulbong	--
		(c) Pultan	---
		(d) Pultin	—
		(e) Ripuk	—
7	Rathan	(a) Tanpok	---
		(b) Tanjong	—
		(c) Tantin	---
		(d) Dasaw	---
		(e) Saring	—
8	Rome	(a) Rungki	—
		(b) Ruling	—
		(c) Rupok	—
9	Tayeng	(a) Subkom	—
		(b) Sunmaw	—
		(c) Kibing	—
		(d) Kirang	.---
		(e) Tonmuk	—-
		(f) Tonkir	- --
		(g) Dasum	—-

Village : Dapui

1. Borang. 2. Irang with two subclans: (a) Numi and (b) Peyang. 3. Legaw.
4. Megu. 5. Perme. 6. Rathan ...... (6 clans).

I5B

TABLE 3—concld.

Village : Mimesipo

1. Borang with four subclans: (a) Damin, (b) Dye, (c) Lischi and (d) Lingyong. 2. Irang with two subclans: (a) Rinbang and (b) Numi. 3. Legaw with two subclans: (a) Limong and (b) Lagrang. 4. Megu. 5. Parthin. 6. Perme. 7. Rathan with three subclans: (a) Tantim, (b) Tanjong and (c) Tanpok . . . . . . (7 clans).

It will be apparent from the above list of clan names (Table 3) that neither the clans nor the subclans are constant in number in all the villages. There are eight clans at Aieng, nine at Mebo, while Dapui and Mimesipo have six and seven clans respectively. It is difficult to explain this variation within such a short distance. The total distance from Aieng to Mimesipo is about 20 miles. Mebo is only 12 miles from Aieng and the former shows a good deal of variation from the latter and the same is seen between Mebo and Mimesipo—a distance of about 8 miles. Mating is, however, preferred within the village than that outside it. Sarma's (1960) data on the Panggi division of the Abor appear to support it as well. According to her, 'all intermarriages take place within the village' and intermarriages do not occur with a neighbouring village only 11 miles away. This will be apparent from our further discussion.

Clans and their population

1. *Clan Borang:* This clan was present in all the four villages. At Aieng it had a total population of 32 souls, 14 males and 18 females. It had three subclans, one of which was further subdivided into two sections (Table 3). At Mebo this clan was divided into five subclans, of which two, Litung and Lingyong, were common to Aieng. At Mimesipo it showed four subclans, of which two, Lischi and Lingyong, were common to Mebo. The last named clan was therefore common to all the above three villages. No subclans of this clan were met with at Dapui. At Mimesipo this clan was recognized as belonging to the Pasi tribe (subtribe ?) of the Abor but at Aieng it was considered to be a Padam clan.

2. *Clan Irang:* As will be obvious from Table 3 this clan was met with in all the four villages but the number of subclans was seven at Mebo and two at each of the other three villages. The two Dapui subclans were common to Mebo, while one of them (Numi) was also met with at Mimesipo. At Aieng the total population of this clan was 29 (males 16; females 13) and of the two subclans, Rinning was reported from Mebo, while Rinbang from the furthest village of Mimesipo.

3. *Clan Legaw:* This clan was reported from all the four villages. It had no subclan at Dapui, while at Aieng four subclans of this clan were met with. It showed a total population of 113 souls (males 60; females 53) at Aieng. All the subclans of Mebo were also reported from Aieng—the

difference was in the Rapok section of the subclan Lagrang being raised to the status of a subclan at Aieng. The Limong subclan was found at Mimesipo only.

4. *Clan Megu:* This clan was also present in all the four villages. It had no subclans at Dapui and Mimesipo, while at the other two villages the names and the numbers of the subclans were similar. The subclan Pebung of Aieng and Nubung of Mebo appeared to be one and the same. At Aieng this clan showed a population of 24 souls, 12 of each sex.

5. *Clan Modi:* This clan was met with at Aieng only and showed a total population of six souls, four males and two females.

6. *Clan Osik:* It was also reported from Aieng only and showed a total population of 14, eight males and six females.

7. *Clan Perme:* It was the second largest clan at Aieng and had a total population of 232 individuals (males 109; females 123). It was reported from the two distant villages of Dapui and Mimesipo but absent at the nearest Mebo. Only three subclans were reported from Aieng.

8. *Clan Tayeng:* This clan showed the largest population of all clans at Aieng (males 141; females 143; total 284). It was also reported from Mebo. All the six Aieng subclans were present at Mebo—the latter having an extra one in Sunmaw.

Besides the above eight clans at Aieng, of which five were common to Mebo, four other clans, Parthin, Rathan, Rome and Lingiligaw, were also met with at Mebo. They were not found at Aieng. Rathan was present both at Dapui and Mimesipo, while Parthin was found at Mimesipo only. All the Rathan subclans of Mimesipo were present at Mebo, there being two more at the latter village. Dapui had no subclans of Rathan. Parthin showed five subclans at Mebo while at Mimesipo it had no subclan.

As already mentioned, it was alleged that there were no Pasi clans at Aieng but the evidences of Mebo and Mimesipo showed that the Borang and the Irang were Pasi clans, present in smaller numbers at Aieng than the Padam ones. The population of each of the Aieng clans is given in Table 4.

Clan Matings

Matings of the two Pasi clans, Borang and Irang, with the other Padam ones will be apparent from Table 5. There were nine matings between Borang and the other Padam clans while in the case of Irang 23 matings were recorded. Interclan matings were also noted and they were as follows:

Tayeng × Tayeng	.. 29
Perme × Perme	.. 19
Legaw × Legaw	.. 1

TABLE 4

Clan population showing the frequency of marrieds and the sex ratio

Sl. No.	Clan	No. of huts	Male	Female	Total	Married			♂ Sex ratio (%)
						Male	Female	Total	
1	Borang	6	14	18	32	7	5	12	43·8
2	Irang	7	16	13	29	6	8	14	55·2
3	Legaw	18	60	53	113	22	24	46	53·1
4	Megu	4	12	12	24	3	4	7	50·0
5	Modi	1	4	2	6	1	1	2	66·7
6	Osik	2	8	6	14	4	4	8	57·1
7	Perme	44	109	123	232	49	58	107	47·0
8	Tayeng	50	141	143	284	70	74	144	49·6
Total		132	364	370	734	162	178	340	49·6

Some of the interclan matings were between the subclans of the clan. At Aieng the clan Tayeng had six subclans while Perme had three only. Whether there is any preferential mating among the different subclans could not be ascertained since the subclan of the wife could not be gathered in all cases. The data are, however, better in respect of the clan Perme, where it was seen that, out of 19 interclan matings, 13 belonged to Mebang × Mekop and one to Mekop · Mai. The rest of the five matings were within the subclan, as follows:

Mebang × Mebang .. 1

Mekop × Mekop .. 4

There was one such mating within the subclan, Tonmuk × Tonmuk, in the Tayeng clan. It is worth while mentioning here that the Perme clan shows the largest excess of females in the village.

TABLE 5

Frequency of clan matings

	Borang	Irang	Legaw	Megu	Modi	Osik	Perme	Tayeng	Total
Borang	—	· ·	—	—	—	—	7	2	9
Irang	1	—·	3	· ·	—	· ·	11	8	23
Legaw	—		1	—	—	· —	11	23	35
Megu	—		—	—·	—	·	1	10	11
Modi ..	—	·	3	· —	· ·	· ·	1	—	4
Osik ..	3	· —	1	· ·	· ·	—	5	7	16
Perme	—	·	—·	—·	· ·	—	19	44	63
Tayeng	· ·	—·	· —	· ·	—	—	—	29	29
Total	4	—	8	—	—	—	55	123	190

Thus the tendency to marry within the same village or within the same clan, which is managed through the different subclans, appears to be an important factor in the Abor mating system. This will be further evident from Table 6, where the frequency of marriages, inside and outside the village, is given in respect of the eight Aieng clans.

TABLE 6

Clans showing marriage distance

Clan	Marriages			
	Total	Within village	Outside village	Unknown
Borang ..	6	3	3	—
%		50·0	50·0	—
Irang ..	7	3	—	4
%		42·9	- --	57·1
Legaw ..	24	13	—	11
%		54·2	—	45·8
Megu ..	5	3	1	1
%		60·0	20·0	20·0
Modi ..	1	—	—	1
Osik ..	3	1	- -	2
%		33·3	—	66·7
Perme ..	81	50	9	22
%		61·7	11·1	27·2
Tayeng ..	88	52	11	25
%		59·1	12·5	28·4
Total ..	215	125	24	66
%		58·2	11·2	30·7

The data of Table 6 are deficient in having a large percentage of ' unknowns ' (30·7 per cent). The high frequency of matings inside the village is borne out by the two largest clans of the village and the Perme clan shows the highest percentage (61·7) of it.

Sex ratio and age structure

The sex and age structure of the different clans of Aieng are given in Table 7. The total population as per the above table is 656 and this variation from the total population of 734 (Table 4) is due to lack of information regarding the age of the individual. Only three clans, Legaw, Perme and Tayeng, show some representative data and when they are compared with the sex ratio for the whole village (49·6 per cent male) a few general remarks are not unjustified.

The age group 0–4 years shows a high male preponderance in all the three clans and the village as a whole. In the next age group 5–9 years,

only the Legaw clan, with the smallest data, shows a male preponderance while in the other three, female preponderance is apparent. The age group, 10–14 years, shows again, like the first age group, high male preponderance in all the four cases. This is again followed by high female preponderance in all the samples between the ages of 15 and 19, excepting the Legaw clan, where the two sexes are equal in numbers. The next age group 20–29

TABLE 7

Sex and age structure of the Aieng clans

Clans	Age groups in years																		Tota
	0–4		5–9		10–14		15–19		20–29		30–39		40–49		50–59		60–69		
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	
Borang	—	2	2	4	1	4	1	3	4	—	1	2	2	1	1	—	—		28
Irang	—	3	3	1	2	1	--	2	7	1	1	2	1	1	—	--	—	.	25
Legaw	6	3	13	8	5	2	3	3	12	12	9	6	4	2	3	3	1	4	99
Megu	1	1	4	1	2	3	1	2	1	1	2	3	1	—	.	1	—	—	24
Modi	--	1	1	---	—	—	--	—	3	—	—	1	—	—	—	—	--	.	6
Osik	3	--	---	1	1	—	—	—	3	2	—	1	1	1	—	---	—	1	14
Perme	15	12	10	22	16	11	4	13	19	19	14	12	17	13	5	3	3	7	215
Tayeng	20	14	18	20	15	10	6	12	20	28	20	11	22	15	5	5	2	2	245
Total Village	45	36	51	57	42	31	15	35	69	63	47	38	48	33	14	12	6	14	656

shows the largest variability in having an equal number of the two sexes in the clans, Legaw and Perme, while the Tayeng clan shows an excess of females in contrast to the male preponderance for the whole village. The next two age groups, 30–39 and 40–49 years, show uniformly the excess of males and the same trend is maintained for the whole village and the Perme clan for the age group 50–59 years as well. The clans Legaw and Tayeng appear to show an equal number of the two sexes for this age group. An excess of females is seen for the highest age group, 60–69 years, in three of the four samples—that of Tayeng only showing an equality of the two sexes.

This picture of the sex ratio is not very much different from the usual rule. It is already well known that there is a high male preponderance in earlier ages, which is nearly equalized during the reproductive period, ending into high female preponderance at the higher age groups. A proper appreciation of the data of Table 7 is not possible without the knowledge of mortality statistics which were very difficult to be gathered from among the Abor since they could hardly say the age at death, beyond such descriptive terms,

as infant, young, adult, old, etc.   The whole village, however, shows a slight excess of females and it is borne out by the two large clans, Perme and Tayeng, and the Pasi clan Borang.   Other clans show a very high male excess excepting the Megu clan, which shows an equality of the two sexes.

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ECOLOGICAL STUDIES OF THE RAJASTHAN DESERT SOILS

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ABSTRACT

Sixty-one soil samples of Rajasthan collected, under different plant communities from different localities, from within the reach of the root system of plants, including both desert as well as semi-desert areas, showed, on analysis, considerable difference in both physical and chemical characters. Such difference varied with the aridity condition of the areas. There are also exceptions, as regards such characters, in respect of Pokaran Rann soils among the desert ones, and the gypsum soils among the other kinds.

INTRODUCTION

Systematic soil studies of the Rajasthan area are scanty at present and whatever little work that has been done so far by Kanitkar (1952), Tamhane (1920) and Raychaudhuri (1952) has been done purely from an agricultural point of view. No attention has been paid so far to the soil from the point of natural vegetation that it bears. Therefore, in our present soil investigations we have tried to give an idea of the conditions of the soil within the reach of the root systems of the natural vegetation.

During our three trips to Rajasthan in all 61 soil samples were collected under different plant communities and from different localities, from the desert and the semi-desert areas the list of which is given in Table I.

These soils were analysed for (1) pH, (2) total soluble salts, (3) chlorides, (4) carbonates, (5) total exchangeable cations and (6) mechanical analysis. Some of the soils were also analysed for water soluble $CaSO_4$ and also for water soluble $MgSO_4$.

1. METHOD

pH was determined in a 1:5 soil water extract electrometrically. Water soluble salts were estimated gravimetrically and chlorides by chromate titration method as given by Piper (1944). The determination of calcium carbonate was carried out by means of Collin's Calcimeter (Wright 1934). Water soluble $CaSO_4$ and $MgSO_4$ were determined by estimating water soluble calcium and magnesium as given by Magistad *et al.* (1945). For determining total exchangeable cations, ammonium acetate neutralized to pH 7 as suggested by Schollenberger (Schollenberger and Simon 1945) was used as the leaching agent and the total cations were determined by Bray and Willhite's method (1929). For mechanical analysis, the dispersion was

TABLE I

Showing analysis of the soils of Rajasthan

Sample No.	Locality	Date	Vegetation cover	Habitat	pH	Total soluble salts %	Chlorides %	Carbonates %	Water soluble CaSO$_4$ %	Water soluble MgSO$_4$ %	Total exchangeable cations m.e.%	Coarse sand %	Fine sand %	Silt %	Clay %	Texture
1	Gadra Rd.	30-6-55	Prosopis spicigera.	Sandy	7·7	0·105	0·010	2·56	0·408	0·086	37·2	63·6	22·7	5·1	4·3	Coarse sand
51	Pokaran	30-10-55	Eliomurus hirsutus.	"	7·9	0·080	0·015	2·48			20·0	44·8	38·3	1·0	2·9	"
52	Jaisalmer	30-10-55	"	"	7·0	0·055	0·015	1·20	0·176	0·009	16·8	58·1	30·2	1·2	6·0	"
58	"	31-10-55	"	"	7·5	0·050	0·015	0·72			15·2	66·1	23·6	0·8	8·9	"
			Average of Eliomurus		7·4	0·061	0·015	1·46			17·3					
59	"	31-10-55	Panicum turgidum.	Sandy	7·3	0·150	0·020	1·52			16·2	59·1	30·2	2·2	3·9	"
68	*Barmer	4-11-55	"	"	7·6	0·040	0·015	1·44	0·272	0·019	28·0	65·2	17·2	5·7	11·4	"
20	Pokaran	10-7-55	"	Rocky	8·0	0·076	0·005	0·24			33·2	52·8	33·0	2·8	2·1	"
			Average of Panicum		7·6	0·051	0·013	1·06			25·8					
10a	*Barmer	4-7-55	Prosopis juliflora and Capparis aphylla.		7·8	0·050	0·015	3·84			24·0	22·8	42·9	13·3	17·8	Sandy loam
33	*Barmer	27-8-55	Prosopis juliflora, Capparis and Calotropis.	Sandy	7·5	0·045	0·010	1·04			19·8	53·7	39·2	1·1	1·1	Coarse sand
			Average of P. juliflora		7·6			2·44			21·9					
54	Chandhan	30-10-55	Calotropis procera.	Sandy	7·8	0·065	0·010	2·00	0·204	0·035	26·4	53·4	30·1	1·7	6·0	"
4	Gadra Rd.	1-7-54	Calotropis procera and Capparis aphylla.	"	7·7	0·055	0·015	2·24			28·0	57·9	31·6	0·9	1·5	"
75	*Barmer	5-11-55	Calotropis procera and Tephrosia purpurea.	"	7·6	0·055	0·013	0·68	0·169	0·035	13·2	66·1	21·0	1·0	5·6	"
			Average of Calotropis on sand		7·7			1·64			22·5					

* These samples are from localities of the semi-desertic region.

TABLE I—contd.

Sam-ple No.	Locality	Date	Vegetation cover	Habitat	pH	Total soluble salts %	Chlorides %	Carbonates %	Water soluble CaSO₄ %	MgSO₄ %	Total exchangeable cations m.e.%	Coarse sand %	Fine sand %	Silt %	Clay %	Texture
74	*Kavas	4-11-55	Calotropis procera.	Gypsum bed.	9.5	0.130	0.015	17.20	0.843	0.020	50.4	29.9	40.5	17.6	9.6	Sandy loam
76	*Kavas	4-11-55	Heylandia latebrosa.	"	8.2	0.140	0.015	2.60	1.044	0.004	39.6	51.8	33.9	5.0	4.8	Coarse sand
78	*Kurla	5-11-55	†Calotropis procera and Aerva pseudotomento-sa.	"				3.20								Coarse sand
80	*Shergarh	6-11-55	Calligonum polygonoides.	Sand-dune	7.8	0.060	0.015	0.32	0.353	0.070	24.0	73.0	23.9	0.8	2.6	"
36	*Barmer	28-8-55	Lycium barbarum.	"	7.4	0.069	0.015	3.68			12.8	42.6	50.3	0.8	2.4	Fine sand
70	Gunga	4-11-55	Capparis aphylla	"	8.6	0.065	0.015	1.76			11.2	40.7	41.1	0.9	3.8	Coarse sand
71	"	4-11-55	"	Saline flat	8.9	0.120	0.015	2.48			20.4	37.7	55.8	0.7	3.0	Fine sand
49	Pokaran	30-10-55	Bouchea marru-bifolia.	Sandy	7.7	0.130	0.015	1.64	0.462	0.038	24.0	43.6	41.6	1.4	2.9	Coarse sand
53	Chandhan	30-10-55	Haloxylon sali-cornicum.	"	7.4	0.050	0.015	1.76	0.408	0.004	24.0	70.8	19.0	0.1	4.9	"
60	Basan Pir Ramgarh	1-11-55	Crotolaria burhia.	"	7.5	0.479	0.015	6.80	0.380	0.032	36.0	35.9	46.3	6.3	6.1	"
65	Jaisalmer	2-11-55	Zizyphus rotun-difolia.	"	8.8	0.065	0.015	1.36			16.0	59.3	34.4	1.0	2.1	"
43	Eumi Jn.	29-8-55	Pluchea lanceo-lata.	Marshy	7.4	0.402	0.010	2.24	0.571	0.043	34.4	50.1	32.8	2.1	5.0	"
61	Lanela	1-11-55	Scirpus quinque-farius.	"	7.6	0.171	0.015	2.96	0.462	0.062	36.0	58.9	28.1	4.0	4.4	"
63	"	1-11-55	Cyperus tuberosus.	"	7.8	0.065	0.015	2.72			20.6	37.6	47.5	0.8	2.2	"
47 / 16	Pokaran	29-10-55 / 8-7-55	Cressa cretica	", Marshy saline.	7.4 / 7.9	0.310 / 0.972	0.015 / 0.465	2.80 / 2.66	0.489 / 0.163	0.038 / 0.019	31.6 / 36.8	44.0 / 38.3	40.9 / 34.1	7.0 / 12.8	3.7 / 10.4	Sandy loam
46	"	20-10-55	(Centre of the lake)	"	6.9	4.532	1.740	4.08	0.843	0.124	36.4	21.4	46.7	15.6	5.2	"
23	*Beriganga	15-7-55	Eleusine flagelli-fera.	Marshy	7.4	0.172	0.007	1.12			22.0	53.0	32.4	5.6	4.8	Coarse sand

* These samples are from localities of the semi-desertic region.

† Sample No. 78, being stony in nature, is omitted from detailed analysis.

TABLE I—contd.

Sample No.	Locality	Date	Vegetation cover	Habitat	pH	Total soluble salts %	Chlorides %	Carbonates %	CaSO₄ %	MgSO₄ %	Total exchangeable cations m.e.%	Coarse sand %	Fine sand %	Silt %	Clay %	Texture
45	Pokaran	29-10-55	Eleusine flagellifera.	Marshy	7·3	0·322	0·015	1·52			17·6	44·7	44·4	1·6	4·6	Coarse sand
66	Fatehgarh	3-11-55	,,	..	8·9	0·065	0·015	1·20	0·462	0·020	14·8	67·0	16·0	7·0	6·6	,,
77	*Uterlai	5-11-55	,,	..	7·6	0·064	0·015	4·40			29·6	22·3	45·6	19·6	6·5	Sandy loam
56	Jaisalmer	31-10-55	Aristida hirtigluma.	Hard packed Sandy	7·4	0·189	0·015	2·80			34·4	42·8	36·3	8·9	7·7	..
50	Pokaran	30-10-55	Aristida hystrix	Sandy	7·6	0·070	0·020	3·36			29·0	53·3	35·8	1·3	2·7	Coarse sand
55	Jaisalmer	31-10-55	Aristida adscensionis.	,,	7·4	0·060	0·005	3·20			39·2	39·5	34·1	7·2	4·8	Sandy loam
64	Basan Pir Raungarh Fatehgarh	1-11-55	,,	Sandy saline	8·5	0·085	0·015	8·72	0·748	0·070	39·6	24·1	36·1	19·7	14·8	..
67	Fatehgarh	3-11-55	,,	Sand-dune	8·7	0·060	0·010	0·88	0·231	0·004	18·4	73·0	15·7	1·6	2·8	Coarse sand
69	Gunga	4-11-55	,,	Rocky hill	8·8	0·020	0·015	0·64	0·462	0·084	11·2	53·3	33·4	0·5	0·5	,,
29	*Mandor	26-8-55	Cenchrus biflorus.	,,	7·4	0·141	0·007	0·56			29·6	21·0	50·9	8·5	6·2	Sandy loam
31	*Barmer	27-8-55	Cenchrus catharticus.	Sand-dune	7·6	0·055	0·015	1·28			19·6	42·6	45·1	1·4	4·3	Coarse sand
34	*Barmer	27-8-55	Cenchrus catharticus and Gynandropsis pentaphylla.	Moist	7·5	0·055	0·015	2·08			12·8	48·8	38·8	0·5	4·7	..
42	*Barmer	28-8-55	Cenchrus catharticus and C. biflorus.	Sandy pasture	7·0	0·060	0·015	0·80			14·0	48·2	40·1	0·4	4·7	..
			Average of Cenchrus catharticus.		7·3	0·056	0·015	1·38			15·4					
26	*Kailana	16-7-55	Acacia senegal	Rocky	7·6	0·060	0·015	1·12	0·380	0·032	35·2	69·9	19·6	4·4	3·0	..
17	Pokaran	9-7-55	Acacia arabica and A. senegal.	Hard	7·5	0·085	0·010	1·20			24·4	39·3	40·3	6·5	4·9	..
62	Basan Pir	1-11-55	Zygophyllum simplex.	Hard saline	7·8	0·115	0·020	8·24	0·978	0·086	44·0	32·3	37·2	21·1	8·5	Sandy loam
57	Jaisalmer	31-10-55	Euphorbia granulata.	Hard pan soil	7·6	0·075	0·015	1·60	0·598	0·435	26·4	56·7	20·9	2·2	4·0	Coarse sand

* These samples are from localities of the semi-desertic region.

TABLE I—concld.

Sample No.	Locality	Date	Vegetation cover	Habitat	pH	Total soluble salts %	Chlorides %	Carbonates %	Water soluble CaSO₄ %	MgSO₄ %	Total exchangeable cations m.e. %	Coarse sand %	Fine sand %	Silt %	Clay %	Texture
73	Fatehgarh	4-11-55	Pennisetum cenchroides var. echinoides.	Hard sandy	7·5	0·060	0·010	3·92			32·8	18·3	47·7	20·3	11·5	Sandy loam
30	*Mandor	26-8-55	Fagonia cretica	Rocky hill	7·4	0·137	0·007	8·00			34·7	34·7	23·3	21·6	10·2	,,
37	*Barmer	28-8-55	Tribulus terristris and Amaranthus blitum.	Dirty	7·6	0·070	0·015	2·32			14·0	40·3	38·9	2·3	4·7	Coarse sand
39	*Barmer	28-8-55	Boerhaavia diffusa.		7·6	0·025	0·015	2·16			28·4	12·6	52·1	6·8	24·6	Sandy clay loam
44	*Luni Jn.	29-8-55	Trianthema pentandra.	Dirty	7·4	0·045	0·010	2·48			22·4	39·2	19·4	9·6	30·2	,,
38	*Barmer	28-8-55	†Digera arvensis	Rocky hill	7·5	0·225	0·015	8·00			28·4	46·4	40·6	4·2	5·6	Coarse sand
10b	*Barmer	4-7-55	,,	Rocky	7·9			1·04								
72	Badka	4-11-55	Wheat cultivation	Sandy	8·9	0·115	0·015	0·65			21·0	45·3	37·0	5·7	4·9	Coarse sand
32	*Barmer	27-8-55	Pennisetum typhoideum (Bajra) cultivation.	,,	7·2	0·063	0·015	0·96			11·6	46·2	44·2	1·6	2·6	,,
35	*Barmer	27-8-55	,, cultivation.	Soil hard	7·5	0·020	0·010	1·52			16·8	38·0	50·4	2·2	3·0	Fine sand
41	*Barmer	28-8-55	Average of P. typhoideum.	Sandy	7·0 7·2	0·044 0·042	0·015 0·012	0·80 1·09			12·0 13·4	50·6	41·0	1·2	1·2	Coarse sand
48	Lathi	30-10-56	†Calcareous stone.					36·8								
11	*Barmer	5-7-55	Salvadora oleoides.	Hard clayey	7·8	0·100	0·030	1·48			19·6	31·6	50·1	6·3	8·5	Fine sand
7	Gadra Rd.	1-7-55	S. oleoides and Calligonum polygonoides.	Sand-dune	8·0	0·050	0·010	2·16			9·6	57·3	32·8	1·0	4·0	Coarse sand

* These samples are from localities of the semi-desertic region.

† Sample Nos. 38 and 48, being stony in nature, are omitted from detailed analysis.

carried out by Troell's method (1931) and for the actual mechanical analysis the method adopted by the Agricultural Education Association (1927-28) was followed. For the mechanical analysis of the gypsum soils, special procedure as described in Robinson's method by Wright (1934) was followed.

2. CHEMICAL FACTORS

(i) *pH*.—It will be seen from Table 1 that the *p*H values of the Western Rajasthan soils vary from 6·9 to 8·9. However, it must be pointed out that on the whole the average values vary from 7·4 to 7·9.

The extreme value of *p*H 6·9 (Sample No. 46 of Table I) is of the soil from Pokaran Rann near Jaisalmer where there was no vegetation cover due to very high salinity (4·53 per cent of total soluble salts). The other extreme value of *p*H 8·9 is of the soils from the region between Badka and Jaisalmer which lies in the extreme desertic area. This is highly significant because, as has been shown in Table II, the chief vegetation of the area is of the sand-dunes covered by *Aristida adscenscionis* and *Capparis aphylla*. As such these species according to Braun-Blanquet (1932) may be called highly basophilous. Apart from these two types of soils with extreme *p*H values, the rest of the soils with *p*H lying between 7·4 and 7·9 may be termed basic or distinctly basic for according to Braun-Blanquet (1932) soils with *p*H between 7 and 7·5 are basic and those with *p*H between 7·2 and 8·5 are distinctly basic.

Therefore, except for the above two, all the soils analysed so far of the Western Rajasthan are basic or distinctly basic.

Species which have a wide range of *p*H are *Elionurus hirsutus* (7·0 to 7·9), *Eleusine flagellifera* (7·3 to 8·9) and *Aristida adscenscionis* (7·4 to 8·8).

*p*H above 8 are shown by the soils of the following samples: (1) Gypsum soils under *Calotropis procera* and *Heylandia latebrosa* (Sample Nos. 74 and 76) from Kavas; (2) *Capparis aphylla* (Sample Nos. 70 and 71) from Gunga; (3) *Eleusine flagellifera* (Sample No. 66) from Fatehgarh; (4) Soil under wheat cultivation (Sample No. 72) from Badka; (5) Soils under *Aristida adscenscionis* (Sample Nos. 64, 67 and 69) from Ramgarh, Fatehgarh and Gunga and (6) Soil under *Zizyphus rotundifolia* (Sample No. 65) from Jaisalmer.

Of the above localities, Fatehgarh, Gunga and Badka fall on the way from Jaisalmer to Barmer and out of nine samples (Sample Nos. 65 to 73) seven show *p*H above 8·6, the two exceptions being Sample Nos. 68 and 73. Though other Jaisalmer and Barmer soil samples show *p*H under 8, the true desertic tract extending from Jaisalmer to Badka, covering Fatehgarh and Gunga, seems to be the region of high *p*H above 8·6. In the semi-desertic area of Barmer the *p*H falls below 8. This fact is made clear by Table II. *p*H values above 7·8 in the semi-desert area are shown only by the Kavas gypsum soils (Sample Nos. 74 and 76 of Table I).

TABLE II

pH of soils from localities lying on the Jaisalmer-Barmer route

Sample No.	Locality	Habitat	Climatic zone	Vegetation	pH
65	Jaisalmer	Sand	Desert	Zizyphus rotundifolia	8·8
66	Fatehgarh	Marshy	,,	Eleusine flagellifera	8·9
67	,,	Sand-dune	,,	Aristida adscensionis	8·7
69	Gunga	,,	,,	,,	8·8
70	,,	,,	,,	Capparis aphylla	8·6
71	,,	Sandy with salt-crustation.	,,	,,	8·9
72	Badka	Sandy, cultivated.	,,	Wheat cultivation	8·9
73	Fatehgarh	Sandy	,,	Pennisetum cenchroides var. echinoides	7·5
68	Barmer	,,	Semi-desert	Panicum turgidum	7·6

(ii) *Salinity.*—According to Sarin (1952), salinity of Rajputana desert is widespread and extensive. The soil is impregnated with saline substances which appear on the surface in the form of white efflorescence, whenever a depression occurs in the ground. The sub-soil is also equally rich in salinity. The salinity varies in nature and chemical composition from place to place but on the average is found to be composed of salts of sodium (chloride), sulphate, carbonate and bicarbonate, and calcium (sulphate). It is generally marked by the absence of magnesium salts and this factor differentiates it from marine salinity. On the whole, the proportion of sodium chloride to the rest of the salts is higher.

Many theories have been put forth to explain the salinity of Rajasthan. Humes suggested that the salt brine of the Rajasthan salt lakes was a mere surface deposit due to drying up of the sea. Allied to this is another theory that below the silt of the lakes or under the mantle of the sand there are old rock-salt beds of marine formation. Geologically both these theories have been proved incorrect. According to Noetling (Sarin 1952) of the Geological Survey of India, the salt of the Sambhar Lake is obtained from the subterranean saline springs. The subterranean percolation from the extensively irrigated areas of the Punjab is offered as another explanation. The generally accepted theory lays down that the salts have been formed by the atmospheric decomposition of granitic and gneissic rocks of the Aravelli hill ranges and leached out by rain water and carried to low-lying lands. Holland and Christie (1909) suggested that the salts were wind borne from

tho Rann of Cutch. This theory has been recently contradicted by Godbole (1952) who has shown that the different salt lakes show different salt compositions.

According to Braun-Blanquet (1932) the saline soils are characterized by higher concentrations of water soluble salts, the common salts being those of chlorides, sulphates and carbonates of sodium, potassium, calcium and magnesium. Therefore, in tho present investigation we havo estimated total water soluble salts, soluble chlorides and carbonates.

(*a*) *Total soluble salts.*—Plants take up mineral matter in the form of soluble salts. The percentage of total soluble salts in soils must, however, be very small. Usually in good soils, total soluble salts do not exceed 0·1 per cent. If the quantity goes higher the plants begin to suffer in proportion to the soluble salts present (Sahasrabudhe 1929).

It is also found that 0·2 per cent of salts in the soil within the range of roots depresses the yield of most of the crops whereas within 0·5 per cent most of them fail. However, crops like *Sesbania aculeata, Triflorum alexandrium* and certain salt-resisting varieties of rice and sugarcane are able to withstand higher salt concentrations and higher alkalinity than pH 9, provided they are well irrigated (Joachim 1941).

Mehta (1937) found that rice yields of about 1,800 kg. per acre are obtained in soils containing not more than 0·2 per cent of salts and having pH not higher than 8·8. He has found that the rise of ground water is an urgent problem and does the greatest harm in sandy soils. As a remedy he suggested the installation of tube-wells as has been done in California and Arizona. He has also given a classification of the alluvial soils of the Punjab in which a soil with 0·2 per cent of salts and pH value 8·5 or less is classed as good land for normal crops and soils with pH above 9·5 as bad and costly to reclaim.

Hoon and Mehta (1937) found that a plant highly tolerant of salts is not a good indicator of the exact soil conditions on which it grows and consequently they studied soil profiles chosen according to the natural vegetation. They found that soils under *Prosopis spicigera, Capparis aphylla, Tamarix auriculata* and *Salvadora oleoides* differed markedly in their soil profiles.

To estimate the salinity of the soils of Rajasthan, a number of soil samples were collected by us from the root region of a number of species and these were analysed for the total water soluble salts. As seen from Table III out of 57 soils analysed, 37 showed total salt content below 0·1 per cent, 14 between 0·1 and 0·2 per cent and only 6 above 0·2 per cent. Therefore, it can be seen that soils in contact with the root systems of most of the plants are non-saline. It is also interesting to note that species like *Eleusine flagellifera* tolerates total soluble salts from 0·064 per cent (Sample No. 77) to 0·322 per cent (Sample No. 45 of Table I).

Highest salinity is shown by Sample Nos. 46 and 16 of the Pokaran Lake with total soluble salts 4·53 per cent and 0·972 per cent respectively. Therefore, the most saline areas are those of the lakes, Sambhar and Didwana as shown by Sarin (1952) and of Pokaran as shown above. As mentioned previously Sample No. 46 had no vegetation at all.

The species which tolerate more than 0·2 per cent of salts are *Crotolaria burhia* (Sample No. 60), *Pluchea lanceolata* (Sample No. 43), *Cyperus tuberosus* (Sample No. 47 from Lanla Rann) and *Cressa cretica* (Sample No. 16 from Pokaran Rann of Table II).

TABLE III

Total soluble salts

Sample No.	Vegetation cover	Per cent
51	Elionurus hirsutus	0·080
52	,,	0·055
58	,,	0·050
20	Panicum turgidum	0·040
59	,,	0·040
80	Calligonum polygonoides	0·060
36	Lycium barbarum	0·069
53	Haloxylon salicoruicum	0·050
63	Cyperus tuberosus and Desmostachya bipinnata.	0·065
7	Salvadora oleoides and Calligonum polygonoides.	0·050
10a	Prosopis juliflora and Capparis aphylla.	0·050
33	Prosopis juliflora, Capparis aphylla and Calotropis procera.	0·050
54	Calotropis procera	0·045
75	Calotropis procera and Tephrosia purpurea.	0·055
4	Calotropis procera and Capparis aphylla.	0·065
70	Capparis aphylla	0·065
66	Eleusine flagellifera	0·065
77	,,	0·064
37	Tribulus terristris and Amaranthus blitum.	0·070
39	Boerhaavia diffusa	0·025
40	Trianthema pentandra and Tribulus terristris.	0·045
32	Pennisetum typhoideum (Bajra) cultivation.	0·064
35	,,	0·020
41	,,	0·044
50	Aristida hystrix	0·070
55	Aristida adscenscionis	0·060
64	,,	0·085
67	,,	0·060
69	,,	0·020
17	Acacia arabica and Acacia senegal	0·085
26	Acacia senegal	0·060
31	Cenchrus catharticus	0·055
34	Cenchrus catharticus and Gynandropsis pentaphylla.	0·055

TABLE III—*contd.*

Sample No.	Vegetation cover	Per cent
42	Cenchrus catharticus and Cenchrus biflorus.	0·060
65	Zizyphus rotundifolia	0·065
73	Pennisetum cenchroides var. echinoides.	0·065
57	Euphorbia granulata	0·075
1	Prosopis spicigera	0·105
11	Salvadora oleoides	0·100
49	Bouchea marrubifolia	0·130
61	Scirpus quinquefarius	0·171
74	Calotropis procera (on gypsum).	0·130
76	Heylandia latebrosa (on gypsum).	0·140
71	Capparis aphylla	0·120
23	Eleusine flagellifera	0·172
72	Wheat cultivation	0·155
30	Fagonia cretica	0·137
56	Aristida hirtigluma	0·189
29	Cenchrus biflorus	0·141
62	Zygophyllum simplex	0·115
68	Panicum turgidum	0·150
16	Cressa cretica	0·972
45	Eleusine flagellifera	0·322
46	Central area of Pokaran lake (no vegetation).	4·532
60	Crotolaria burhia	0·479
43	Pluchea lanceolata	0·402
47	Cyperus tuberosus	0·310

Cressa cretica is the most salt tolerant species for it grows in soils containing soluble salts as high as 0·972 per cent.

In the Pokaran Rann four distinct zones of vegetation were observed. The outermost was formed by *Eleusine flagellifera*, the next by *Cyperus tuberosus* and *Demostachya bipinnata*, the third by *Cressa cretica* and *Aeluropus villosus* and the fourth by a bare central portion. The conditions prevailing in each of the four zones are given in Table IV.

The changes in the salt content of each of the four zones is distinct. *Cressa cretica* and *Aeluropus villosus* tolerate salts up to about 1·0 per cent. However, the former was observed to be more dominant than the latter. In the central portion of the Rann the soluble salt is 4·5 per cent and too toxic to support any plant life.

In Table IV, the total soluble salts, carbonate and the exchangeable cations, on the whole, show gradual increase from Zone I to Zone IV. However, it will be noted that this is not true for the pH values.

Hence it seems that of all the above factors, the total soluble salts and in turn the chloride content seem to be the guiding factors affecting the vegetation of the Rann.

TABLE IV

Showing four zones of vegetation in the Pokaran Rann

Zone No.	Vegetation	pH	Total soluble salts %	Chlorides %	Carbonates %	Total exchangeable cations m.e.%	Coarse sand %	Fine sand %	Silt %	Clay %	Soil texture
I	Eleusine flagellifera	7·3	0·322	0·015	1·52	17·6	44·7	44·4	1·6	4·6	Coarse sand
II	Cyperus tuberosus and Desmostachys bipinnata.	7·4	0·310	0·015	2·80	31·6	44·0	40·9	7·0	3·7	Coarse sand
III	Cressa cretica and Aeluropus villosus.	7·9	0·972	0·465	2·66	36·8	38·3	34·1	12·8	10·2	Sandy loam
IV	No vegetation	6·9	4·532	1·740	4·08	36·4	21·4	46·7	15·6	5·2	Sandy loam

Finally it may be noted that the clay and silt fractions show increase from Zone I to Zone IV and the soils become more and more sandy loamy.

Our values of total soluble salts as compared with those of the Cutch Rann soils (Agricultural Education Association 1927-28) are very low. Whereas the values for the latter range from 3 to 17 per cent (Table V) the highest values for the Pokaran Rann is 4·53 (Sample No. 46 of Table III) with an average of only about 0·1 per cent for the rest of the soils.

The relation of the texture of the soil to the total soluble salts is shown by Tamhane (1920). According to him those soils having a sandy substratum do not have high values of total soluble salts for all the salts are leached out through this porous layer and they cannot rise again to the surface layer for sand has a poor capillary power to raise these salts. Those soils having a clayey layer show high percentage of soluble salts and that is why the need of sandy substratum for the reclamation of saline lands is stressed.

From the analysis of our soils given in Table 1 it will be observed that our results bear out the above relationship because most of the Rajasthan soils are highly sandy containing from 58 to 91 per cent of coarse and fine sand but the total soluble salts are on the average only about 0·1 per cent. On the other hand, the soils of the Rann of Cutch which are mostly clayey in texture with 12 to 62 per cent of clay show total soluble salts ranging from 3 to 17 per cent as shown in Table I.

Weaver and Clements (1938) have pointed out that all the soil salts except the carbonates of sodium and potassium are neutral in reaction and do not affect the pH. Sarin (1952) has shown that in the lake soils of Sambhar and Didwana potassium carbonate is altogether absent and sodium carbonate is negligible. Since our results given in Table I show no relationship between pH and the total soluble salts, it is presumed that the carbonates of sodium and potassium are absent or negligible in the Western Rajasthan soils.

(b) *Chlorides.*—The chloride content of the Rajasthan soils ranges from 0·01 to 0·15 per cent except for those of the Pokaran Rann which show values from 0·46 to 1·74 per cent (cf. Sample Nos. 16 and 46 of Table I). As such the chlorides do not seem to have influence on the total soluble salts. As has been pointed out earlier in Table I, the soils of the Pokaran Rann where the chloride content rises as high as 1·74 per cent, the vegetation is extremely poor *or* nil.

Mehta (1937) has given a classification of the alluvial soils of the Punjab in which a soil with 0·2 per cent of salts and pH value 8·5 or less is classed as good land for natural crops and soils with pH above 9·5 as bad and costly to reclaim.

TABLE V

Analysis of the soils of the Rann of Cutch

Place	Locality and Remarks	pH	Total soluble salts %	Water soluble CaSO₄ %	Water soluble MgSO₄ %	Coarse sand %	Fine sand %	Silt %	Clay %	Soil texture according to Weir (1936)
(1) Gauthana (profile)	Sea water approaches this area	8·4	7·29	3·230	—	21·3	1·8	45·1	31·8	Clay
(2) Nanda (profile)	River water which turns saline after admixture with sea water floods the area.	7·9	10·92	1·306	—	10·4	8·0	49·0	41·6	Clay
(3) Lakhpat-Piprala (profile)	No sea water but flood water rendered saline inundates this area.	7·7	8·68	1·034	0·110	6·0	22·8	23·1	48·1	Clay
(4) Bela (profile)	Thick surface deposit of salt and sea water sometimes approaches this area.	7·7	12·31	0·910	—	5·7	11·0	20·8	62·5	Clay
(5) Kavda-Barren area (profile)	Two areas nearby, one with and one without salt.	8·3	8·86	2·145	—	2·6	69·1	15·5	12·8	Sandy loam
(6) Kavda-Camel Bush area (profile)	One area with camel bush vegetation and one with coarse grass.	7·8	3·11	0·479	0·184	Nil	40·2	29·7	30·0	Clay loam

*After Satyanarayan (1951).

Table I shows that in most of the soils the total soluble salts are less than 0·2 per cent and their pH values are under 8·5 and they never exceed 8·9.

Therefore, these Rajasthan soils may be taken as good lands for normal crops.

(iii) *Calcium carbonate.*—In its effect upon the distribution and growth of plants the bivalent Ca ion ($^{++}$) is second only in ionic effect to hydrogen-ion (H^+). Calcium, of which manifold importance in plant structure is well known, is found extensively in nature as humate in organic combination, as a sulphuric salt (Gypsum), as a silicate but most abundantly in the form of a carbonate. The effectiveness of the calcium content for plants is determined by its solubility rather than by the absolute amount of calcium present. Calcium carbonate, being an easily soluble compound, is always accessible to plants. It affects in large measure the physico-chemical conditions of the soils and thereby indirectly the vegetation. Soils rich in lime have a neutral or alkaline reaction, since calcium carbonate neutralizes acids (Braun-Blanquet 1932).

According to Braun-Blanquet (1932) plants which thrive in a dry climate are more and more confined to calcareous soils as they approach the northern limits of their ranges, since those soils alone can afford them favourable physical conditions of temperature, water supply and soil aeration. Further he states that many communities of the arid regions are lime-constant in the strictest sense.

Our soil analysis presented in Table I reveals that the Rajasthan soils are rich in calcium carbonate and the value is usually 1 to 3 per cent. Soils with more than 1 per cent carbonates are considered as rich and with more than 3 per cent as calcareous by Braun-Blanquet (1932).

Only the following samples show carbonates less than 1 per cent:—

Sample No.	Plant cover	Percentage carbonates
80	Calligonum polygonoides	0·32
58	Elionurus hirsutus	0·72
75	Calotropis procera and Tephrosia purpurea	0·68
32	Pennisetum typhoideum (Bajra) cultivation.	0·96
41	,,	0·81
67	Aristida adscenscionis	0·88
69	,,	0·64
42	Cenchrus biflorus and Centhrus catharticus.	0·80
29	Cenchrus biflorus	0·56

However, of the above, other soil samples of *Elionurus hirsutus, Calotropis procera, Pennisetum typhoideum* (Bajra) cultivation, *Cenchrus catharticus,* and *Aristida adscenscionis* show more than 1 per cent of carbonates.

The species under which the carbonate content is exceptionally high (more than 4 per cent) are the following: --

Sample No.	Plant cover	Percentage carbonates
* 77	Eleusine flagellifera	4·49
* 64	Aristida adscenscionis	8·72
60	Crotolaria burhia	6·80
* 62	Zygophyllum simplex	8·24
46	Pokaran lake (no vegetation).	4·08
38	Digera arvensis	8·00
30	Fagonia cretica	8·00

However, *Aristida adscenscionis* and *Eleusine flagellifera* from other places do not show these high carbonate values.

Another point of interest for the soils mentioned above with an asterisk mark is that they show a crustation on their surface which was mistaken for chlorides. The total salt and chloride contents of these soils are poor and this crustation seems to be of carbonates and not of the chlorides.

A similar crustation was found under *Capparis aphylla* near Gunga village (Sample No. 71) but in this sample the carbonate is only 2·48 per cent which is slightly higher than that of Sample No. 70 of *Capparis aphylla* where there was no such crustation and the carbonate was 1·76 per cent.

The carbonate content of the soil of *Calotropis procera* on gypsum (Sample No. 74) is exceptionally high, 17·20 per cent. However, the value of the carbonates is not so high in other gypsum soil samples (Nos. 76 and 78). In the former it is 2·60 per cent and in the latter 3·20 per cent. In this connection Braun-Blanquet (1932) states that gypsum soils are rather rich in carbonates and further adds that according to an author though the gypsum bed rock contains no calcium carbonate, in the weathered gypsum rock 10 per cent or more of calcium carbonate may be present. Therefore our results of gypsum soils confirm the above statement.

The most exceptional value of carbonates was shown by the analysis of the calcareous stones which contained as much as 36·8 per cent of $CaCO_3$.

Therefore, it may be concluded that the soils are rich in calcium carbonate and the plant associations are lime-constant as stated by Braun-Blanquet (1932). Our results also agree with those of Ghosh (1952) who has shown that the desert sand contains 1·13 per cent to 7·63 per cent calcium carbonate.

(iv) *Water soluble $CaSO_4$ and $MgSO_4$.*—As compared to Cutch Rann soils, some of our soils estimated for $CaSO_4$ are poorer. The values in the Rann of Cutch soils as seen from Table I vary from 0·479 to 3·230 per

cent, whereas in the Rajasthan soils values range from 0·163 to 1·044 per cent (cf. Table I).

Higher values in Rajasthan soils are shown by the Kavas gypsum soils (Nos. 74 and 76) ranging from 0·843 to 1·044 per cent.   The soils with surface crustation as Sample No. 62 of *Zygophyllum simplex*, No. 64 of *Aristida adscenscionis* and No. 46 of the Pokaran Lake show comparatively high figures of $CaSO_4$ (0·700 to 0·900 per cent).   There seems to be a positive correlation between water soluble $CaSO_4$ and calcium carbonate for it is found that the higher the $CaSO_4$, the higher are the carbonates.

Water soluble magnesium sulphate is present in very small quantities 0·004 to 0·124 per cent in the Rajasthan soils and is comparable with the Rann of Cutch soils where the value ranges from traces to 0·184 per cent. Generally the higher the value of $CaSO_4$, the higher is the value of $MgSO_4$.

(v) *Total exchangeable cations.*—The importance of exchangeable cations as an index of soil fertility has now fully been recognized.   Chemical analysis of the soils do not show the relative proportions of available and unavailable forms of most elements.   Hence as far as the basic nutrients are concerned, the determination of exchangeable bases, held by colloids, forms a much more significant measure of soil fertility.

Exchangeable cations have an important effect on the general soil properties and its behaviour.

In the Rajasthan soils as shown in Table I the total exchangeable cations vary from 9·6 to 50·4 m.e. per cent, the average value being 24·0 m.e. per cent. The highest value of 50·4 m.e. per cent is shown by Sample No. 74, under *Calotropis procera*.   Compared to the soils of the ruderal vegetation of Bombay (Jindal 1956; Meher-Homji 1955) which vary from 14 to 49 m.e. per cent and to the grassland soils (Nileshwar 1956) which vary from 5 to 49 m.e. per cent, the Rajasthan soils may be said to be equally rich in spite of their sandy nature.   This can be explained on the fact stated by Raychaudhuri and Sen (1952) that the present soil cap of the Western Rajasthan being formed under arid climate is expected to contain all the nutrient elements.

3.   Physical Factors

Mechanical Analysis

Weir (1936) has classified soils on the structural basis and on this basis the Rajasthan soil seems to fall under the following four groups: (1) Coarse sand; (2) Fine sand; (3) Sandy loam and (4) Sandy clay loam.

Out of 58 soil samples analysed for mechanical structure (1) 41 show coarse sand, (2) 3 fine sand, (3) 2 sandy loam and (4) 2 sandy clayey loam texture.

The species that occur on coarse and fine sand are *Elionurus hirsutus*, *Panicum turgidum*, *Prosopis spicigera*, *Bouchea marrubifolia*, *Haloxylon salicornicum*, *Calotropis procera*, *Crotolaria burhia*, *Zizyphus rotundifolia*, *Calligonum polygonoides*, *Lycium barbarum*, *Capparis aphylla*, *Cyperus tuberosus*, *Scirpus quinquefarius*, *Pluchea lanceolata*, *Aristida hystrix*, *Cenchrus catharticus*, *Pennisetum typhoideum* (Bajra) and *Triticum vulgare* (wheat); those found on sandy loam are *Cressa cretica*, *Aristida hirtigluma*, *Cenchrus biflorus*, *Zygophyllum simplex* and *Pennisetum cenchroides* var. *echinoides*; those that occur on sandy clay loam are *Boerhaavia diffusa* and *Trianthema pentandra*.

Aristida adscenscionis occurs on three types of soils, namely on highly sandy sand-dunes, on coarse sandy soils and also on sandy loamy soils (cf.

TABLE VI

Showing plants in relation to their observed habitat and soil texture

Plants	Observed habitat	Soil texture
Prosopis spicigera	Loose sandy	Coarse sand
Elionurus hirsutus	,,	,,
Panicum turgidum	,,	,,
Bouchea marrubifolia	,,	,,
Haloxylon salicornicum	,,	,,
Calotropis procera	,,	,,
Crotolaria burhia	,,	,,
Zizyphus rotundifolia	,,	,,
Aristida hystrix	,,	,,
Cenchrus catharticus	,,	,,
Wheat cultivation	,,	,,
Pennisetum typhoideum cultivation.	,,	Coarse and fine sand
Salvadora oleoides	,,	Fine sand
Calligonum polygonoides	Sand-dunes	Coarse sand
Lycium barbarum	,,	Fine sand
Aristida adscenscionis	Sand-dunes and sandy plains	Coarse sand and sandy loam
Capparis aphylla	,,	Coarse sand and fine sand
Calotropis procera	Gypsum soil	Sandy loam
Heylandia latebrosa	,,	Coarse sand
Acacia senegal	Rocky	,,
Aristida histigluma	,,	Sandy loam
Cenchrus biflorus	,,	,,
Fagonia cretica	,,	,,
Zygophyllum simplex	Hard substratum	,,
Pennisetum cenchroides var. echinoides.	,,	Coarse sand
Cyperus tuberosus	Marshy	,,
Scirpus quinquefarius	,,	,,
Pluchea lanceolata	,,	,,
Cressa cretica	,,	Sandy loam
Eleusine flagellifera	,,	Coarse sand and sandy loam
Boerhaavia diffusa	Nitrophilous, ruderal soils	Sandy clay loam
Trianthema pentandra	,,	,,
Tribulus terristris	,,	Coarse sand

Sample Nos. 55, 64, 67 and 69 of Table I) and *Eleusine flagellifera* on two types, namely the coarse sand and the sandy loam (Sample Nos. 23, 45, 66, and 77).

From the above statement it is apparent that all the soils of Rajasthan contain high proportion of either coarse or fine sand. This is in contrast with the soils of the Rann of Cutch (Satyanarayan 1951) which as can be seen from Table V are generally clayey in texture for out of 6 samples analysed, four are clayey, one clayey loam and the remaining sandy loam. Therefore in texture our soils are different from those of the Rann of Cutch. On the other hand, the soils of Western Rajasthan are akin to those of the Thar-Parkar divisions of Sind with 85 to 95 per cent sand and 5 to 7 per cent clay, according to Tamhane (1952) and Rathore (1950). Our results also agree with those of Raychaudhuri (1952) who has shown the top layers of the soils of Ajmer to be sandy with 3 to 8 per cent clay.

The relation between the soil texture and the soluble salt content is already pointed out previously.

In Table VI is given an account of plants in relation to their observed habitat and soil texture.

4. COMPARISON OF THE SOILS OF THE DESERT AND SEMI-DESERT REGIONS

From the analysis of the soils for different chemical and physical factors the following comparison can be made between the extreme dry region called the desertic lying between Badka and Jaisalmer and Pokaran and Jaisalmer and the semi-desertic region lying east of Badka up to Jodhpur.

As seen from Table VII the first contrasting factor is the pH. The soils of the desertic region lying between Badka and Jaisalmer show pH values varying from 8·5 to 8·9, i.e. all these soils are highly alkaline. In this connection it may further be pointed out that the only exception to the desert soils is the soil of the fourth zone of the Rann of Pokaran near Jaisalmer which, as previously mentioned, shows pH 6·9 and is too saline to bear any vegetation. On the other hand the soils of the semi-desertic region show much less pH values, namely from pH 7 to 7·9. However, here again there is exception in the gypsum soils of Kavas which show pH of 8·2 to 8·5, which are almost as high as those of the first region.

In total soluble salts and in chlorides the soils of the two regions do not seem to differ much, the average respective values being 0·10 and 0·01 per cent for the first region and 0·13 and 0·01 per cent for the second. Once again the soils of the Pokaran Rann are an exception, the respective values for the total soluble salts and chlorides being 0·97 to 4·53 per cent and 0·46 to 1·74 per cent.

In carbonates, average values of the soils of the two regions once again differ, those of the desert region have an average of 2·4 per cent and those of

TABLE VII

Analysis of the soils of the desert and the semi-desert area

A. The desert area

Sample No.	Date	Place	Plant cover	Habitat	pH	Total soluble salts %	Chlorides %	Carbonates %	Total exchangeable cations m.e. %	Mechanical analysis				Soil texture
										Coarse sand %	Fine sand %	Silt %	Clay %	
1	30-6-55	Gadra Rd.	Prosopis spicigera	Sandy Plain ground	7.7	0·105	0·010	2·56	37·2	63·6	22·7	5·1	4·3	Coarse sand
2	1-7-55	,,	Calotropis procera, Capparis, Salvadora		7.7	0·065	0·010	2·24	28·0	57·9	31·6	0·9	1·5	,,
7	1-7-55	,,	Salvadora oleoides and Calligonum	Sand-dune	8·0	0·050	0·010	2·16	9·6	57·3	32·8	1·0	4·0	,,
16	8-7-55	Pokaran	Cressa cretica	Dry marshy	7·9	0·972	0·465	2·66	36·8	38·3	34·1	12·8	10·4	Sandy loam
17	9-7-55	,,	Acacia arabica and A. senegal	Hard clayey	7·5	0·085	0·010	1·20	24·4	39·3	40·3	6·5	4·9	Coarse sand
2)	10-7-55	,,	Panicum turgidum	Rocky hill	8·0	0·040	0·005	0·24	33·2	52·8	33·0	2·8	2·1	,,
47	29-10-55	,,	Cyperus tuberosus	Marshy	7·4	0·310	0·015	2·80	31·6	44·4	40·9	7·0	3·7	,,
45	29-10-55	,,	Eleusine flagellifera	Marshy	7·3	0·322	0·015	1·52	17·6	44·7	44·4	1·6	4·6	Sandy loam
46	29-10-55	,,	,,	Centre of saline lake	6·9	4·532	1·740	4·08	36·4	21·4	46·7	15·6	5·2	,,
49	30-10-55	,,	Bouchea marrubifolia	Saline	7·7	0·130	0·015	1·04	24·0	43·6	41·6	1·4	2·9	Coarse sand
50	30-10-55	,,	Aristida hystrix	Sandy	7·6	0·070	0·020	3·36	29·0	53·8	35·8	1·3	2·7	,,
51	30-10-55	,,	Elionurus hirsutus	,,	7·9	0·080	0·015	2·48	20·0	44·8	38·3	1·0	6·0	,,
52	30-10-55	,,	,,	,,	7·0	0·055	0·015	1·70	16·8	58·1	30·2	1·2	4·9	,,
48	30-10-55	Lathi	Calcareous stones					36·80						
53	30-10-55	Chandhan	Haloxylon salicornicum	Saline	7·4	0·050	0·015	1·76	24·0	70·8	19·0	0·1	4·9	,,
54	30-10-55	,,	Calotropis procera	Sandy	7·8	0·045	0·015	2·00	26·4	53·4	30·1	1·7	6·0	,,

TABLE VII—contd.

Sample No.	Date	Place	Plant cover	Habitat	pH	Total soluble salts %	Chlorides %	Carbonates %	Total exchangeable cations m.e. %	Coarse sand %	Fine sand %	Silt %	Clay %	Soil texture
55	31-10-55	Jaisalmer	Aristida adscensionis	Sandy	7·4	0·060	0·005	3·20	39·2	39·5	34·1	7·2	4·8	Sandy loam
56	31-10-55	,,	Aristida hirtigluma	Hard	7·4	0·189	0·015	2·80	34·4	42·8	36·3	8·9	7·7	,,
57	31-10-55	,,	Euphorbia granulata	Hard pan soil	7·6	0·075	0·015	1·60	26·4	56·7	20·9	2·2	4·0	Coarse sand
58	31-10-55	,,	Eliomurus hirsutus	Sandy	7·5	0·030	0·015	0·72	15·2	66·1	23·6	0·8	8·9	,,
59	31-10-55	,,	Panicum turgidum	,,	7·3	0·040	0·020	1·52	16·2	59·1	30·1	2·2	3·9	,,
65	2-11-55	,,	Zizyphus rotundifolia	,,	8·8	0·065	0·015	1·36	16·0	59·3	34·4	1·0	2·1	,,
61	1-11-55	Lenela	Scirpus quinquefarius.	Marshy	7·6	0·171	0·015	2·96	36·0	88·9	28·1	4·0	4·4	,,
63	1-11-55	,,	Cyperus tuberosus	Dried marshy	7·8	0·065	0·015	2·72	20·6	37·6	47·5	0·8	2·2	,,
60	1-11-55	Basan Pir, Ramgarh	Crotolaria burhia	Sandy	7·5	0·479	0·015	6·80	36·0	35·9	46·3	6·3	6·1	,,
62	1-11-55	Basan Pir, Ramgarh	Zygophyllum simplex	Hard gravelly and saline	7·8	0·115	0·020	8·24	44·0	32·3	37·2	21·1	8·5	Sandy loam
64	1-11-55	Fatehgarh	Aristida adscensionis	Saline	8·5	0·085	0·015	8·72	39·6	24·1	36·1	19·7	14·8	,,
66	3-11-55	,,	Eleusine flagellifera	Dried marshy	8·9	0·065	0·015	1·20	14·8	67·0	16·0	7·0	6·6	Coarse sand
67	3-11-55	,,	Aristida adscensionis	Sand-dune	8·7	0·060	0·010	0·88	18·4	73·0	15·7	1·6	2·8	,,
73	4-11-55	,,	Pennisetum cenchroides var. echinoides	Gravelly	7·5	0·060	0·010	3·92	32·8	18·3	47·7	20·3	11·5	Sandy loam
69	4-11-55	Gunga	Aristida adscenscionis	Sand-dune	8·8	0·020	0·015	0·64	11·2	53·3	33·4	0·5	0·8	Coarse sand
70	4-11-55	,,	Capparis aphylla	,,	8·6	0·065	0·015	1·76	11·2	40·7	41·1	0·9	3·8	,,
71	4-11-55	,,	,,	Saline	8·9	0·125	0·015	2·48	20·4	37·7	55·8	0·7	3·0	,,
72	4-11-55	Badka	Wheat cultivation	Sandy	8·9	0·115	0·015	0·65	21·0	45·3	37·0	5·7	4·0	,,

TABLE VII—contd.

B. The semi-desert area

Sample No.	Date	Place	Plant cover	Habitat	pH	Total soluble salts %	Chlorides %	Carbonates %	Total exchangeable cation m.e. %	Mechanical analysis				Soil texture
										Coarse sand %	Fine sand %	Silt %	Clay %	
23	15-7-55	Beriganga	Eleusine flagellifera	Marshy	7·4	0·172	0·007	1·12	22·0	53·0	32·4	5·6	4·8	Coarse sand
26	16-7-55	Kailana	Acacia senegal	Rocky hill	7·6	0·060	0·015	1·12	35·2	69·9	19·7	4·4	3·0	,,
29	26-8-55	Mandor	Cenchrus biflorus	,,	7·4	0·140	0·007	0·56	29·6	21·0	50·9	8·5	6·2	Sandy loam
30	26-8-55	,,	Fagonia cretica	,,	7·4	0·137	0·007	8·00	37·6	34·7	23·3	21·6	10·2	,,
31	27-8-55	Barmer	Cenchrus catharticus	Sand-dune	7·6	0·055	0·015	1·28	19·6	42·6	45·1	1·4	4·3	Coarse sand
32	27-8-55	,,	'Bajra' field	Sandy	7·2	0·064	0·015	0·96	11·6	46·2	44·2	1·6	2·6	,,
33	27-8-55	,,	Prosopis juliflora and Capparis	,,	7·5	0·050	0·010	1·04	19·8	53·7	39·2	1·1	1·1	,,
34	27-8-55	,,	Cenchrus catharticus and Gynandropsis	Moist	7·5	0·055	0·015	2·08	12·8	48·8	38·8	0·5	4·1	,,
35	27-8-55	,,	'Bajra' field	Compact and hard	7·5	0·020	0·010	1·52	16·8	38·6	50·4	2·2	3·0	Fine sand
36	28-8-55	,,	Lycium barbarum	Sand-dune	7·4	0·069	0·015	3·68	12·4	42·6	50·3	0·8	2·4	,,
37	28-8-55	,,	Tribulus terristris and Amaranthus blitum	Dirty	7·6	0·070	0·015	2·32	14·0	40·3	38·9	2·3	4·7	Coarse sand
38	28-8-55	,,	Digera arvensis	Rocky hill	7·5	0·025	0·015	8·00	28·4	12·6	52·1	6·8	24·6	Sandy clay loam
39	28-8-55	,,	Boerhaavia diffusa	,,	7·6	0·044	0·015	2·16	12·0	30·6	41·0	1·2	1·2	Coarse sand
41	28-8-55	,,	'Bajra' field	Sandy	7·1	0·060	0·015	0·08	14·0	48·2	40·1	0·4	4·7	,,
42	28-8-55	,,	Cenchrus catharticus and C. biflorus	Sandy	7·0	0·060	0·015	0·80	14·0	48·2	40·1	0·4	4·7	,,
10a	5-7-55	,,	Prosopis juliflora	pasture	7·8	0·051	0·010	3·84	24·0	22·8	42·9	13·3	17·8	Sandy loam
10b	4-7-55	,,	Prosopis juliflora and Capparis	Rocky	7·9	0·225	0·015	1·04	28·4	46·4	40·6	4·2	5·4	Coarse sand

TABLE VII—concld.

Sample No.	Date	Place	Plant cover	Habitat	pH	Total soluble salts %	Chlorides %	Carbonates %	Total exchangeable cations m.e. %	Mechanical analysis				Soil texture
										Coarse sand %	Fine sand %	Silt %	Clay %	
11	5-7-55	Barmer	Salvadora oleoides	Hard clayey.	7.8	0.100	0.030	1.48	19.6	31.6	50.1	6.3	8.5	Fine sand
68	4-11-55	"	Panicum turgidum	Sandy.	7.6	0.150	0.015	1.44	28.0	65.2	17.2	5.7	11.4	Coarse sand
75	5-11-55	"	Calotropis procera and Tephrosia purpurea.	"	7.6	0.055	0.015	0.68	13.2	66.1	21.0	1.0	5.0	"
43	29-8-55	Luni Jn.	Pluchea lanceolata	Near the river.	7.4	0.402	0.010	2.24	34.4	50.1	32.8	2.1	5.0	"
44	29-8-55	"	Trianthema pentandra.	Dirty	7.4	0.045	0.010	2.48	22.4	39.2	19.4	9.6	30.2	Sandy clay loam
77	5-11-55	Uterlai	Eleusine flagellifera	Marshy	7.6	0.064	0.015	4.40	29.6	22.3	45.6	19.6	6.5	Sandy loam
80	6-11-55	Shergarh	Calligonum polygonoides.	Sand-dune.	7.8	0.060	0.015	0.32	24.0	73.0	23.9	0.8	2.6	Coarse sand
74	5-11-55	Kavas	Calotropis procera	Gypsum	8.5	0.130	0.015	17.20	50.4	29.9	40.5	17.6	9.6	Sandy loam
76	5-11-55	"	Heylandia latebrosa	"	8.2	0.140	0.015	2.60	30.6	51.8	33.9	5.0	4.8	Coarse sand
78	5-11-55	Kurla	Calotropis procera and Aerva pseudotomentosa.	"				3.20						

the semi-desert less than 2 per cent. The Pokaran soils are once again an exception, their values on the whole being less than 2·4 per cent. The gypsum soils of the semi-desert region are also an exception in that they show extreme high values rising up to 17·0 per cent.

In total exchangeable cations the soils of the semi-desert region are on average equally divided between high and low values, they being respectively 50 and 11·6 m.e. per cent. The highest values are shown by the soils of the rocky hills and of Kavas (i.e. gypsum soils). The desertic soils also show low and high values but in this case the proportion between the two is 2 : 3. The highest values are shown by the soils of the Pokaran Rann and the soil of Ramgarh under *Zygophyllum simplex*. On the whole the lowest values are shown by the sandy and the sand-dune soils.

In mechanical structure, the soils of the two regions differ considerably. Whereas those of the desert region may be classified into two groups of Weir (1936), namely (1) Coarse sand and (2) Sandy loam, those of the semi-desert region may be classified into four groups of Weir, namely (1) Coarse sand, (2) Fine sand, (3) Sandy loam and (4) Sandy clay loam, i.e. the desert soils are on the whole more sandy than those of the semi-desert.

Thus it will be seen that the desert soils differ considerably in their chemical and physical factors from the semi-desert soils. Further the Pokaran Rann soils are always an exception to the soils of the desert region and the gypsum soils to those of the semi-desert region.

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SUPER-, MULTI- AND HYPERPARASITISM AND THEIR EFFECT ON THE BIOLOGICAL CONTROL OF INSECT PESTS

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ABSTRACT

The last decade and a half will ever be remembered, in the annals of applied entomology, as the period when some of the most powerful insecticides ever known to man were synthesized. Yet DDT, gammexane, the organic phosphorous compounds and other chlorinated hydrocarbons, while solving some problems, have also created newer and more difficult problems. So, the biological basis of insect control has again come to the forefront. But the introduction and establishment of beneficial parasites from one country into another is no easy matter. If failures are to be avoided, there should be sustained research on all aspects of the problem. In this paper, the complex phenomena of super-, multi- and hyperparasitism are unfolded against the general background of insect parasitism on which the biological control of insect pests rests.

INTRODUCTION

Though the biological basis of insect control is one of the most effective and economical methods of bringing under subjugation insect pests of agricultural and horticultural crops, the latter half of the present century can be rightly called an age when organic insecticides and weedicides rule the mind of entomologists all over the world. This in the main could be attributed to certain historical reasons.

Wigglesworth (1950), in a notable address delivered at the annual meeting of British Association for the Advancement of Science, observed that the discovery of lead arsenicals and paris green in the latter half of the nineteenth century to control the Colorado beetle, *Leptinotarsa decemlineata* Say., a serious pest of potato sweeping across the two continents—America and Europe—was a landmark in the story of applied entomology. Since then entomologists and chemists have always dreamt of an ideal insecticide that would not only kill the pest, but also leave a residue on or in the plant body to fight successfully insect invasions. In this context, the discovery of DDT as an insecticide about the year 1939 was another milestone in the history of chemical warfare against insect pests. Indeed, the last decade and a half will ever be remembered in the annals of applied entomology as the period when some of the most powerful insecticides ever known to man were synthesized. Yet DDT, gammexane, the organic phosphorous compounds and other chlorinated hydrocarbons, while solving some problems, have also created newer and more difficult problems. The widespread and injudicious application of insecticides covering

large areas have resulted in the destruction of predators and an upset in the balance of population. On account of the destruction of the predatory species the injurious pests that were kept in check by these predators and pests have multiplied in geometrical progression causing devastation to large areas of agricultural and horticultural crops. For instance, mites that were not considered as pests of agricultural and horticultural crops a decade ago have assumed the status of major pests and are sweeping across the continent of America. This is on account of the destruction of predatory species that are more susceptible to organic insecticides than the phytophagous species. The large-scale intensive application of insecticides has also resulted in the appearance of resistant forms that may eventually prove very difficult to control. Lastly, these organic insecticides have also raised the important question of hazards to the health of human beings and livestock. As Leland Cole (1950) observes: ' Although even today the food crops treated against insect pests are only a small fraction of the total protection, the increasing use of insecticides mostly more or less toxic to man has raised queries about their effect on health.' On account of these new problems that have been created by the synthesization of organic insecticides, the biological basis of insect control has again come to the forefront and entomologists of America, where most of the modern organic insecticides were synthesized and used, have again begun a world-wide search for beneficial parasites and predators and are combing every country that lies in the temperate, sub-tropical and tropical zones. As Munro (1929) observes: ' Insecticides as a class are palliative measures bringing about a more or less rapid decrease of the pests during the first or second season of its abundance.' Wigglesworth (1950) goes a step further when he says: ' By all means let us use insecticides when we can do no better. But we should regard them as C. B. Williams has emphasized more than once as an admission of failure to be replaced by the more subtle and more remunerative methods of biology as soon as these can be worked out.' There can be little doubt that the biological basis of insect control is certainly one of subtle and remunerative methods. Of this method Munro says: ' More fundamental in its application is the control measure termed " parasite control ".' But the introduction and establishment of parasites from one country into another is no easy matter. Again as Munro (1929) remarks: ' It must in fact be admitted that some of the early successes in parasite introduction were largely fortuitous and we realize today that the chances of successful introductions are not always good but on the contrary failures should be frequent.' If failures are to be avoided, if parasite introductions should be successful, there should be sustained research, more and more research to probe into every aspect of the problem, like superparasitism, multiparasitism, hyperparasitism, specificity, prolificacy, etc. Two of these factors on which the success or failure of biological control experiments rests are super- and multiparasitism. To this, the fascinating

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phenomenon of hyperparasitism is closely linked.   Indeed hyperparasitism is a dominant factor in the maintenance of a subtle balance between the host and the primary parasites.   In the following pages, the complex phenomena of super-, multi- and hyperparasitism are unfolded against the general background of insect parasitism on which the biological control of insect pests is based.

SUPERPARASITISM

Definition of term :

Superparasitism has been defined by Fiske (1910) as a phenomenon ' where an individual host is attacked by two or more species more than once '.   The definition as given above by Fiske clearly embraces two distinct phenomena: one phenomenon where the individual host is simultaneously attacked by two or more species of primary parasites and the other where the individual host is attacked by the single species of primary parasite more than once.   Pierce (1908) gave the former phase of parasitism the term ' accidental secondary parasitism '.   The same author (1910) later designated these two phases as mixed superparasitism (his earlier secondary parasitism) and cannibal superparasitism.   Smith (1916), in an attempt finally to redefine some of the aspects of relationships between host and the parasite, limited the use of the term superparasitism ' to that form of symbiosis occurring where there is a superabundance of parasites of a single species attacking an individual host insect more than once '.   He also restricted the use of the term multiparasitism to that form of symbiosis ' where the same individual host insect is infested simultaneously with the young of two or more different species of primary parasites '.   Haviland (1922) uses the term ' hyperparasitism ' to describe this phenomenon though the term is used in quite a different sense by entomologists in England and America.   The English and the American entomologists consider Smith's definition as the most satisfactory one and in this review it is used in the sense as defined by him.

Superparasitism is a very common occurrence in the parasite families of Chalcidoidea and Braconidae (Hymenoptera) and all the parasite individuals successfully complete their development and eventually emerge as adults. Imms (1937) states that superparasitism in many cases is a normal and regular occurrence and results in no fatal effect upon the parasite progeny.   In the hymenopterous families of Platygasteridae, Encyrtidae, Braconidae and Dryinidae where the phenomenon of polyembryony occurs, superparasitism is obligatory and a number of individuals numbering sometimes hundreds and even thousands may emerge from a single host.   For instance, it has been observed that as many as 3,000 individuals of a species of a polyembryonic chalcid have been reared from one individual *Plusia* larva.   Where superparasitism is not obligatory, its occurrence is due to the result of either of

errors of instinct or of competition induced by a scarcity of suitable hosts. In such cases, fatal consequences commonly intervene. Timberlake (1912), in giving complete data for only one of his experiments, has shown that, out of 19 parasites distributed among 17 hosts, two hosts remained unparasitized, 12 contained one each, two contained two each and one three parasites. Here is an example to illustrate the inborn instinct of the mother to distribute her progeny.

Fiske (1910) found that ' the prevalence of superparasitism depends entirely upon whether or not the female parasite is gifted with a prescience which will enable her to select healthy hosts for her offspring. Presupposition that she possesses this prescience is equivalent to a denial of the existence of super-parasitism. This is indubitably not in accordance with conditions as they exist in the field and laboratory'. He developed a mathematical treatment and concluded that parasite eggs are distributed at random among the members of the host. Thompson (1924) supported the theory of Fiske and mathematically expressed the phenomenon in the following formula $Y = N \left(1 - e - \dfrac{x}{N} \right)$, where N represents the number of hosts, X the number of parasite eggs, Y the number of hosts parasitized and e the Napierian logarithmic value. But later on many workers like Salt (1934) and Jackson (1939) showed by experimental evidence that the theory of random distribution was not applicable to all parasites. Salt (1934) has clearly shown that the chalcid *Trichogramma* does not distribute her progeny at random.

Superparasitism and host selection :

Salt (1934) has worked on various aspects of the problem. It is apparent that host selection is one of the main factors involved in the occurrence of superparasitism. Salt (1934) observes : ' Every parasite has a number, one or more, of host species which it will attack. The inclusion in the number of some species, and the exclusion from it of others, implies a process of selection on the part of the parasite which is usually known as host selection.' A parasite chooses certain hosts, from a large population available to it, is a well-known fact; but why it chooses those particular individuals and rejects others has not been clearly understood. Even after selection, why should a parasite lay more than one egg on a single host even though a large number of hosts not parasitized are available ? It is evident that more than a single factor is involved in this problem of host selection. Some of these are:

(1) Behaviour of the parasite.
(2) Choice of the host and distribution of progeny.
(3) Vigour of the parasite.
(4) Size of the host.
(5) Restraining and discriminating power of the parasite.

Each one of these factors is reviewed in detail below:

(1) *Behaviour of the parasite*

The high selective faculty of the female parasite which enables her to choose the best of the available hosts depends upon the behaviour of the particular parasite in relation to the particular host.   Flanders (1935) has observed that the larger specimens tend to ignore hosts of less volume than their own bodies.   Salt (1935) has observed that while the female *Trichogramma* hardly took about five seconds to examine and reject a parasitized host egg, the same parasite required 15 to 30 seconds to accept a healthy egg.   Recently Narayanan, Subba Rao and Kaur (1959) studied the oviposition response and host selection in *Apanteles angaleti* Muesebeck.   In this instance, the host selection was based primarily on physical characters of the host, like the toughness or rigidity of the integument.   Stimulus for oviposition was the sense of touch and not that of smell.   Superparasitism occurred where a suitable host was available.

(2) *Choice of the host and distribution of progeny*

Lloyd (1938) while studying the factors governing the choice of hosts and distribution of progeny by the chalcid, *Ooencyrtus kuvanae* Howard, observed that the female parasite usually selected unparasitized hosts and when forced to superparasitize, chose the hosts that contained the youngest parasite stage. Narayanan and Chacko (1957) studied the phenomenon of superparasitism in *Trichogramma*.   These studies revealed that in this parasite the instinct to oviposit soon after emergence and almost immediately after mating was so great that it did not even leave off a host egg after oviposition and oviposited in the same egg again and again with the result that superparasitism automatically resulted.   The urge for oviposition was so great in this parasite that it laid two or three eggs in a host during a single insertion of the ovipositor of the parasite.   Further they observed that the progeny that emerged from highly superparasitized host eggs were unable to mate with the healthy ones.   The occurrence of superparasitism in the case of *Trichogramma* was dependent more or less on the number of host eggs available rather than the period of exposure. Ullyett (1949) observed that *Microbracon hebetor* Say. deposited eggs in batches in periodic acts of oviposition presumably because the parasite had first to paralyse the host larvae and then return to oviposit on them.

Leiby and Hill (1923) observed that *Platygaster hiemalis* Forb. laid eggs in groups of 4 to 8 at each thrust of the ovipositor in the host Hessian fly.   The eggs developed monoembryonically and polyembryonically and in the latter case twin adults were formed.   They also observed that ·when *Platygaster vernalis* (Meyrs) attacked the Hessian fly, on an average eight adults emerged from a single parasite egg.

Miller (1959) studied the host parasite relationship of the spruce bud-worm, *Choristoneura fumiferana* (Clem.), and its parasite, *Apanteles fumiferanae* Viereck, both in the laboratory and in the field. The low incidence of super-parasitism in the laboratory and the occurrence of a high degree of super-parasitism in the upper crown of the tree suggested that the searching capacity of the female parasite was certainly not at random. His studies also suggested that the parasite, guided by certain sensory mechanism, caused it to (a) discriminate between the healthy and parasitized hosts, (b) go to the upper level of the crown, and (c) choose foliated spruce over heavily defoliated fir tree. The relation between the degree of attack and host density was not well defined except where more than one species of host food plant was considered and in this instance factors other than host density confounded the relationship.

Guppy (1959) found that superparasitism always occurred in the case of *Euplectrus melippes* Prov., a larval parasite of the army worm, *Pseudoletia unipuncta* (Haw.). This ecto-parasite was reared from the fourth and fifth *instar* larva, the number of parasite larvae ranging from 5 to 21, the average being 8.

Salkeld (1959) while studying the oviposition behaviour of *Aphaereta pallipes* (Say.), a braconid parasite of the onion maggot, *Hylemya antiqua* (Meig.), observed that apparently the female was attracted towards the host larvae on account of the strong odour of the onion, but ultimately discovered its host by a sense of touch. Nearly 60 parasitic eggs had been dissected out from a single host maggot although the maximum number of adults that emerged from a host puparium was only 30. It was also found that one female could deposit only 20 eggs in a single host.

(3) *Vigour of the parasite*

It is a well-known fact that the more vigorous and active a parasite is, the more longer and faster can it fly to locate its host. Narayanan *et al.* (1948) in their studies on the host selection of *Bracon gelechiae* found that, in cases where more than one parasite grub shared a single host, the size of the individual parasite decreased and in certain cases degenerate forms or runts appeared as a result of overcrowding and consequent insufficiency of food during the developmental period.

Flanders (1935) observed that smaller specimens of *Trichogramma* were unable to parasitize the eggs of *Pachysphinx modesta* which were readily parasitized by larger ones. Salt (1940) also made the same observation when he used specimens of *Trichogramma* bred from various lepidopterous eggs. Invariably larger parasites, that emerged from *Baratha* eggs, parasitized hosts more quickly than the medium-sized individuals bred from *Ephistia* eggs, which in their turn were quicker than those individuals bred from *Sitotroga* eggs which were the smallest.

(4) *Size of the host*

Salt (1940) was able to demonstrate experimentally the discriminatory faculty of *Trichogramma* to choose between the small and the large eggs. It was also noticed that *Trichogramma* invariably superparasitized the larger eggs.

(5) *Restraining and discriminating power of the parasite*

Salt (1934) has done some remarkable work on the phenomenon of super-parasitism in *Trichogramma*. This small wasp not only discriminates the healthy from the parasitized host eggs but is also able to locate the host eggs parasitized by other *Trichogramma*. This ability is due to *perception* and not due to *memory*.

However, Lloyd (1935) thinks that the discriminatory factor in *Tricho-gramma* is of a qualitative nature rather than quantitative. Salt (1934) concludes from his experiments that superparasitism increases when the number of host eggs available is limited; if only parasitized eggs are available or exposed, *Trichogramma* refrains from superparasitizing by retaining her eggs. In several cases the occurrence of superparasitism is not due to the absence of discriminative faculty of the parasite, but due to its inability to refrain from depositing her eggs in hosts which have been parasitized.

Ullyett (1936) observed that maturation of ova within ovaries of the gravid female gave a psychological urge which makes a female search not only a suitable host but also a stadium in the life cycle of the host. The search for the host must be within the reach of the female depending upon the vigour and strength to fly long distances and also in a limited time. When once the eggs are mature, they have to be deposited within a certain period, depending upon the age and condition of the parasite. During this period of search, if the female gets only a limited number of hosts, superparasitism intervenes.

Ullyett (1949) studied the distribution of progeny by *Cryptus inornatus* Pratt and came to the conclusion that the occurrence of a high degree of super-parasitism at low host density did not necessarily mean that the parasite had lost its faculty to eliminate superparasitism. It implied rather that, when host material was scarce, the psychological urge for depositing mature eggs was overwhelmingly predominant, and forced the female to utilize hosts which were available, whether they were parasitized or not, although under more favourable circumstances she would normally avoid doing so. The partial breakdown in the basic psychological factors resulted in superparasitism and gave a wrong inference of partial random oviposition. The efficiency of the parasite was governed by a number of factors, including those of an inherent psychological nature which was produced by the condition of the reproductive system at any given time.

Ullyett (1950) has made a comparative study of the oviposition behaviour and distribution of progeny in three species, viz. *Cryptus*, *Chelonus* and *Bracon*. *Chelonus*, an egg-larval parasite, mainly depends upon a favourable ratio of hosts to parasite, in order to attain its reproductive efficiency. The females are very prolific and the egg production is continuous throughout their reproductive phase. Oviposition, therefore, is affected more or less uninterruptedly as long as host material is available within the universe inhabited by the parasite. On the other hand *Bracon hebetor* females are comparatively prolific and the daily egg production is high. Unlike *Chelonus*, *Bracon* attacks moving hosts which must be paralysed first and then oviposit on it. The search for hosts and the act of oviposition apparently take place in two distinctive phases. The deposition of eggs is periodical and batches of eggs are presumably ready for laying at certain intervals. *Bracon* lays more than one egg per host, several progeny usually being able to complete their development on each host larva. The parasite is apparently able to judge the size of the host in relation to the number of progeny it can support to maturity and to adjust the number of eggs laid per host accordingly, a faculty which is very necessary in this type of parasitism in order to prevent the intervention of true superparasitism.

Superparasitism : Fecundity, longevity and sex ratio

Narayanan *et al.* (1948) in their studies on superparasitism in *Bracon gelechiae* Ashmead observed that superparasitism had a direct bearing on (*a*) the size of the individual bred, (*b*) the number of adults bred and (*c*) the sex ratio of the offspring. Narayanan and Chacko (1957) studied the phenomenon of superparasitism in *Trichogramma* and its effect on the progeny. They observed that as a result of superparasitism, though one to three adults might emerge from the egg of *Corcyra cephalonica* St., they were defective; they were generally inactive and possessed ill-developed wings. They mated with forms of about their own size only, if they showed any tendency to mate at all; and even if they mated, the fecundity was very low. Their observations led to the conclusion that superparasitism was a very potent factor that determined the success or failure of the colonization of *Trichogramma* in the field.

In many cases the sex ratio depends upon whether the eggs had fertilized or not at the time of oviposition.

Yasumatsu Seizo (1953) while studying the reproductive capacity of *Aicetus ceroplastis* Ishi, an effective parasite of *Ceroplastes rubens* Maskell in Japan, observed that the vigour of the virgin female was as good as that of a mated parasite, the difference being that the virgin female produced only parthenogenetically.

Narayanan and Subba Rao (1955) studied the phenomenon of superparasitism in *Bracon gelechiae* Ashmead and its effect on the sex ratio in detail.

CHART I

Regression line for the sex ratio on the number of grubs per host.
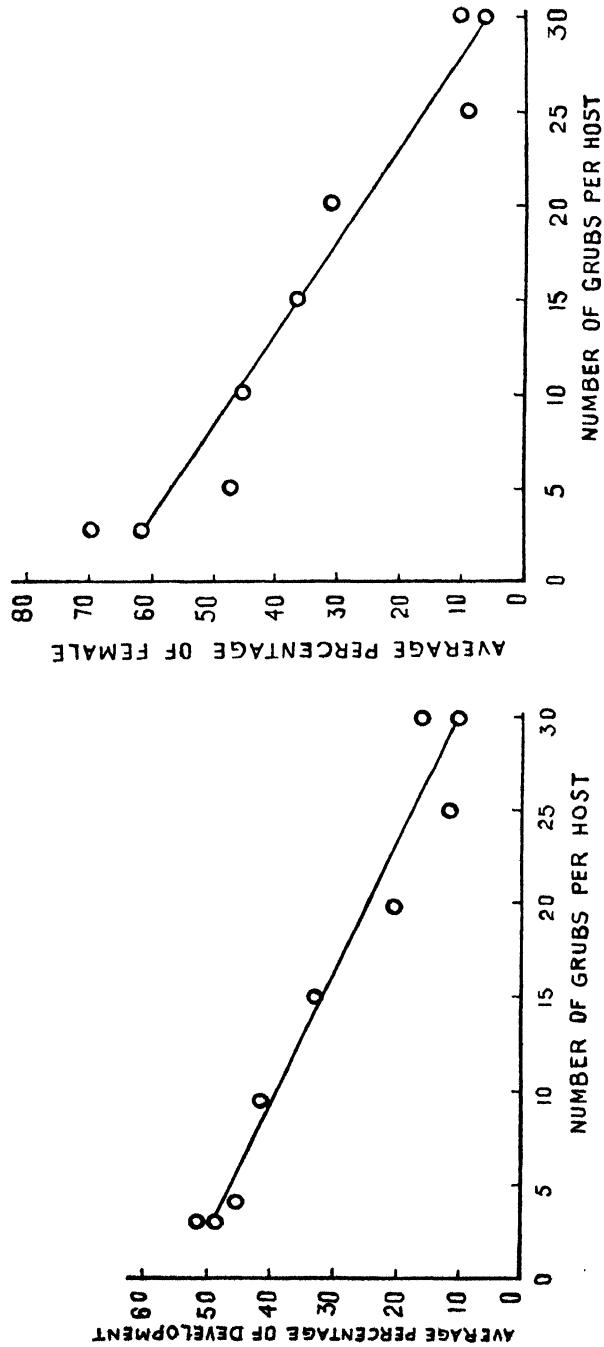
Regression line for the percentage of development on the number of grubs per host.

(After Narayanan, E. S., and Subba Rao, B. R., *Beitr. Zur. Ent.*, 5 (1-2), 36–60)

They concluded that the production of excess of males in many cases was the direct effect of inadequate external stimulation of the spermatheca which depended on the host suitability. In gregarious species like *Bracon gelechiae* the male sex dominated because of differential mortality resulting from superparasitism. From their studies it was evident that the haploid male was less affected by larval starvation than deploid males and females. They substantiated their critical experimental studies by a regression line.

MULTIPARASITISM

The term 'multiparasitism' is used to designate that type of parasitism in which the same individual insect is inhabited simultaneously by the young of two or more different species of primary parasites.

The phenomenon of multiparasitism is well known and is of common occurrence in nature. Very often the female parasite of two different species cannot distinguish between a parasitized and unparasitized host and so results in multiparasitism. In other words its occurrence would appear to be dependent upon errors of instinct on the part of the ovipositing female. This phenomenon also obtains in many cases as the result of acute competition induced by a high ratio of parasites to host population, where oviposition in at least a part of the universe of already parasitized individuals becomes unavoidable.

The biological implication of multiparasitism was first pointed out by Howard (1897) in his excellent study of the parasitism of the tussock moth. In this paper he stated that 'many parasites suffer from the rivalry based upon erroneous instinct'. He did not, however, undertake to determine the causes for this competition on the group efficiency of the parasite but his statement that 'it is unwise and most unpromising to attempt heterogeneous and miscellaneous importations of parasites without careful study of the host insect on its home ground and in its natural environment throughout the whole range of its existence and a similar biological study of its parasites and natural enemy under such conditions' was of considerable importance.

Fiske (1910) another pioneer worker on insect parasitism called attention to this question although, in his discussion of the subject, he did not clearly distinguish between 'super- and multi-parasitism', which from an economic point of view was an entirely distinct phenomena.

The idea that two parasites competing for the same host insect might actually result in less destruction of the host than one of them alone would have accomplished, was first advanced by Pemberton and Willard (1918). They discussed on the interactions of several species of hymenopterous parasites introduced into· Hawaii by a worker for the control of the Mediterranean fruit fly, *Ceratitis capitata* Weid., all attacking the same stage of host. They concluded from their studies 'sufficient evidence has been presented to

prove that superiority of the parasite *Opius humilis* over the other introduced fruit fly parasites in Hawaii and demonstrates the decided restraint operated over it by the unfailing cannibalistic activities of the larvae of *Diachasma tryoni* in particular and of the other parasites in part.   Knowing the capacity of *Opius humilis* for parasitizing 60 to 90 per cent of the larvae of fruit fly in favourable localities such as the Kona coffee belt on the island of Hawaii, the authors here maintain that detrimental results to a certain extent have arisen from the liberation in Hawaii of parasites other than *Opius humilis*.   The total parasitism has simply been reduced in value to that of a parasite of secondary value '.   This was a matter of practical significance and it brought extensive changes in most biological control programmes.

Smith (1929) has critically analysed this problem and concludes that it is far from proven that the effects of multiparasitism would have been less beneficial than activities of *Opius* alone.   He stresses the superior balancing effect of several species of parasites over a single species in that, that they are not all alike to suffer in the same degree of the various deleterious factors. Several species would tend to stabilize a parasite population whereas a single species would tend to fluctuate in efficiency only annually.   From the data of Pemberton and Willard (1918) where one phase of problem is represented graphically, Smith (1929) claims that even if 17 per cent of the puparia which were attacked by both species produced *Opius* alone, it was extremely doubtful whether they would have had the effect of increasing the parasitism by *Opius* from 48·1 to more than 65·1 per cent.

CHART II
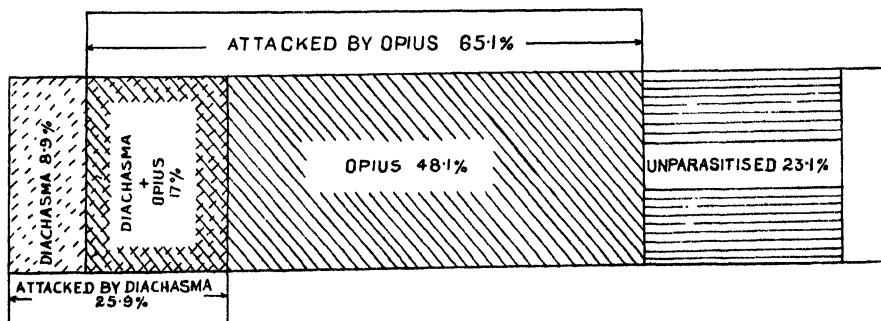
(After Smith, H. S. (1929). *Bull. ent. Res.*, 20, 141–49)

MANIFESTATION OF THE PHENOMENON

Several types of competition may occur between two parasites attacking the same individual host and it may simplify its phases of multiparasitism, if they are defined and separated into different categories for the benefit of

research workers. Smith (1929) suggested that the behaviour of the parasites might differ in two ways, viz. (a) in relation to each other and (b) in relation to their environment. The two parasites might be equally fitted to survive the competition with the host, the issue being decided solely by the time of attack or by some other factor not characteristic of the parasites themselves or one species specially fitted to survive because of certain specific qualifications such as superior mouth parts or greater aggressiveness or because it secretes some toxic substances fatal to its rival or for some other reason. In order to distinguish between the two, Smith called the types 'Intrinsically superior' and 'Intrinsically inferior' parasites. In relation to their environment he has also grouped the parasites into categories. The tendency of parasites, as with other organisms, is to maintain the higher average population and the population is largely determined by the environment. As between two parasites, for example, one may have a higher saturation point than the other which Smith has designated as 'Extrinsically superior' and 'Extrinsically inferior' parasites. On the basis of the above findings, Smith has separated the following types of manifestations of multiparasitism:

(1) Neither species is intrinsically or extrinsically superior.
(2) One species is intrinsically superior, but neither species has any extrinsic superiority.
(3) One species is extrinsically superior but neither has any intrinsic superiority.
(4) One species is both extrinsically and intrinsically superior.
(5) One species is intrinsically superior but extrinsically inferior.

Phases of multiparasitism

The phases of multiparasitism are various. Imms (1937) has classified them into three categories.

(a) *Multiparasitism involving the survival of a single species of parasite*

The occurrence of this type appears to be very prevalent and the survivor may bring about the death of the other parasite or parasites either by direct attack or by indirect effects of its presence. The studies of Pemberton and Willard (1918) on the interrelations of fruit fly parasites in Hawaii have shown that when the braconids *Diachasma tryoni* Cam. and *Opius humilis* Silv. occur together in the same host, the latter species was usually killed and the former developed to maturity. *Opius humilis* is killed purely by wounds and lacerations inflicted upon it by the long-curved sickle-like mandibles of the newly-hatched larva of *Diachasma tryoni*. Besides possessing unusual power for inflicting injury to other parasitic larva about it, it invariably avoids counter-attack through its ability to manoeuvre quickly and also through the protection offered to it by a thick mass of serosal cellular material around

CHART III

Sequence of parasites of hyphantria in 1913

Parasites	Egg stages		Larval stages							Pupal stages				Adult
	Fresh	Old	1st	2nd	3rd	4th	5th	6th	7th	Pre-pupa	Fresh	Old	
Apanteles													
Meteorus													
Campoplex I													
Campoplex II													
Ernestia													
Therion													
Pimpla													

(After Tothill, *Tech. Bull.*, *3* (*N.S.*), *Dept. Agric. Ottawa*)

its body that accompanies the larva when it emerges from the egg and remains around it during its entire life in the first *instar*. They dissected a total number of 627 fruit fly puparia and found that 143 contained larvae of the two species of parasites and that in 133 of these the *Opius* larvae were found to have been destroyed.

Pierce (1910) in his paper on insect enemies of weevils enumerates a number of examples of multiparasitism; the surviving species is recorded where known, but whether it gained the ascendency by direct attack upon its competitor or by indirect nutritional effects was apparently not determined.

In other cases of multiparasitism a passive struggle for existence results and the death of one or the other species supervenes upon exhaustion of the food supply by the survivor. As a rule those parasites which attacked a given host earlier reached the destructive feeding stage first and were consequently survivors in the struggle. Thus Tothill (1922) in discussing the parasites of *Hyphantria cunea* mentions that one caterpillar which he dissected contained 22 parasitic larvae belonging to four species; all the parasites were alive but at least 21 of them were doomed to an early death. The possibilities of this struggle for existence among the competing parasites can be seen by a glance at Chart III in which the horizontal lines represent the stage of host during which the various parasitic larvae are found.

By reading through the columns one can see the possibilities for multiparasitism in the caterpillars of different stage larva. For instance, one could expect only to find *Apanteles* and very occasionally *Therion*. In a single second-stage larva one might find an *Apanteles*, a *Meteorus*, a *Campoplex* and a *Therion*. Likewise various combinations of parasites occur in the third stage also. In the course of many thousands of dissections that have been made, there is not a single instance where all the possible parasites have been harboured in a single third-stage caterpillar, though there is little doubt that instances of the kind occasionally occur in nature. Four species were actually found together in one larva and a number of host larvae were found to contain three species.

Another noteworthy example is instanced by Muesebeck (1918) in his studies of the parasites of the brown-tail moth *Euproctis chrysorrhoea* L. As a result of dissections of over thirteen thousand host caterpillars, it was found that whenever larvae of the braconid *Apanteles lacteicolor* entered into competition with those of *Meteorus versicolor* Wesm., or with the tachinid *Zygobothria nidicola* Towns., or with both species, the latter parasites were killed evidently as a result of some toxic secretion induced by *Apanteles*. Varley (1947), while working on the natural control of population of the knapweed gall-fly *Urophora jaceana*, observed that the mortality of gall-fly due to parasitism in August and September, 1935, was invariably rather too low. Cases of multiparasitism were observed only before the host was completely

consumed by an ectoparasite. The ectoparasitic larvae of the Chalcids *Torymus*, *Cyanimus*, *Habrocytus* and *Macroneura* might destroy the endo-parasitic *Eurytoma curta* with that of its host. Varley also noted that the outcome of such competition depended very much on the timing of the attack. If the *Eurytoma* larva had caused the pupation of the fly larva before the eggs of the other parasites had hatched, then the newly-hatched ectoparasitic larvae died of starvation, being unable to bite through the hard puparium and so the *Eurytoma* larva survived.

The successive rise to predominance by three of the most successful parasites of the oriental fruit fly *Dacus dorsalis* Hendel. in Hawaii has been an interesting development in the biological control of this pest. Van Den Bosch and Haramoto (1953) reported that the first species to gain prominence was *Opius longicaudatus* (Ashmead), a parasite of the second and third *instar* fruit fly larva, which increased in abundance rapidly after its introduction in the summer of 1948. It spread throughout the inlands and was recovered in abundance for about a year. *Opius vandenboschi* Fullaway, which attacks only the first *instar* fruit fly larva, then replaced *O. longicaudatus* in prominence. *O. vandenboschi* was first liberated at about the same time as *O. longicaudatus*. The former parasite though scarce in the beginning became abundant until the summer of 1950, and was then suddenly replaced by *O. oophilus* Fullaway, an egg larval parasite. Ever since *O. oophilus* replaced *O. vandenboschi* the former appeared destined to continue to be the one established parasite. Apparently multiple parasitism existed among the three introduced parasites. However, the interrelationship of the three species was interesting. The data collected indicated that a few *Opius longicaudatus* larvae were able to survive in competition with *Opius vandenboschi* in the same host, but in most instances, the *O. longicaudatus* larvae died. The first *instar* of *O. vandenboschi* possessed strong mandibles, was very active and invariably killed the weakly mandibulate and sluggish *O. longicaudatus*. The interrelations of *O. oophilus* and *O. longicaudatus* were studied and the same type of relationship was found to exist. However, *O. oophilus* possibly had a slightly stronger inhibitory effect on the hatching of *O. longicaudatus*. But the interrelationship between *O. oophilus* and *O. vandenboschi* was entirely different. *O. oophilus* attacked fruit fly eggs and *O. vandenboschi* parasitized early first *instar* larvae. Ninety host larvae were found to contain eggs or larvae of both the species. None of the *O. vandenboschi* eggs, found in these host larvae, showed evidence of development. In many cases the dead *O. vandenboschi* eggs showed consid-erable internal disorganization and were usually in a collapsed state.

The inhibitory effect of *Opius oophilus* and *O. vandenboschi* appeared to be physiological, since no visible injury to the eggs was observed and in the early stage of degeneration the eggs were turgid and normal in every respect except for the internal disorganization of yolk.

Narayanan, Subba Rao and Kaur (1959) studied the phenomenon of multiparasitism in the leaf miner *Phytomyza atricornis* (Meig.) by the eulophid *Rhopalotus* sp. and a braconid (now described as *R. thakarei* Subba Rao and *Opius* sp.) both being endoparasitic. Invariably the eulophid *Rhopalotus* sp. completed the development and the braconid succumbed to the effects of multiparasitism. The authors pointed out that the stronger braconid was killed on account of lack of oxygen available in the host rather than through other means.

Subba Rao (1957) while studying the biology and bionomics of *Lestodryinus pyrillae* Kieffer, a nymphal parasite of *Pyrilla perpusilla* Walker, observed the occurrence of multiparasitism between *Lestodryinus pyrillae* and the strepsipteran *Pyrilloxenus compactus* Pierce. The Dryinid *L. pyrillae* always developed and attained maturity while the strepsipteran *P. compactus* died.

(b) Multiparasitism without parasite mortality

This phase is frequently met with among the parasites of lepidopterous larvae and lecaniine scales. In the case of *Coccus caprea*, the hawthorn scale, multiparasitism is a common phenomenon. The encyrtid parasites *Blastothrix sericea* and *Aphycus punctipes* have been reared from an individual host scale in Britain. Though there is successful development of both the species, the effects of competition upon the insects concerned are usually in proportion to the number present. The most obvious result is the development of individuals stunted or reduced in size, and material reduction in their productive capacity.

(c) Multiparasitism involving the death of parasites

Instances of this kind have been seldom recorded but it appears that the fatal consequences are the result of exhaustion of food supply or of the premature death of the host. In his studies of the parasites of the white marked tussock moth, Howard (1897) observed that when the ichneumon *Pimpla inquisitor* oviposited in caterpillars already parasitized by a tachinid, both the parasitic grubs invariably died. Pierce (1910) found that in the case of the parasites of weevils survival was rare when more than two species of parasites were involved.

<div align="center">

HYPERPARASITISM

</div>

In addition to super- and multiparasitism there is a far more complex phenomenon occurring in nature.

> ' Big fleas have little fleas upon their back to bit 'em
> And little fleas have lesser fleas and so *ad infinitum* '.

'Hyperparasitism' according to Smith (1929) is generally used to denote any stage of parasitism other than primary. Comparatively few primary parasites of a given insect are immune from attacks by their own specific parasites. According to Imms (1937) the largest number of secondary parasites are members of the ichneumon subfamily Cryptinae and Ophioninae and the superfamily Chalcidoidea. A large number of proctotrupoids and cynipids also add to the list of hyperparasites. Bombylidae among Diptera contain some species which are also known to be secondary parasites.

It is not possible to classify, absolutely, certain species as primary parasites or secondary parasites since their behaviour is elastic and largely determined by the types of hosts adopted. Timberlake (1913) found that the chalcid *Coccophagus lecanii* Fitch, a common primary parasite of the soft scale, *Coccus hesperidum*, when in association with other primary parasites like *Microterys* and *Aphycus*, became a hyperparasite. *Monodontomerus aereus* Walker and *Eupteromalus nidulans* (Forst.) occur as primary parasites of the Gipsy moth and brown tail moth respectively, but both the species also frequently become hyperparasites through braconid or tachinid parasites of their hosts.

Hyperparasites in general are far less restricted in their host selection than most hymenopterous primary parasites. They are able to adopt themselves to a wider range of hosts, especially in the absence of preferred hosts—a trait which explains the heavy attacks that certain introduced primary parasites undergo in countries where normal and regular hyperparasites are absent. Narayanan, Subba Rao and Rao (1957) gave an example of a remarkable parasite complex in the larva of *Hymenia recurvalis* F. The caterpillars were attacked by three species of primary parasites of which *Apanteles delhiensis* Muesebeck and Subba Rao was the most abundant. A series of hyperparasites belonging to the families Elasmidae, Miscogasteridae, Eurytomidae, Eupelmidae, Eulophidae and Pteromalidae of Chalcidoidea and Ceraphronidae of the Proctotrupoidea was reared in the laboratory from the field collected *Apanteles* cocoons. It was also remarkable that from each pupa only one particular species of hyperparasite emerged. In other words there was no multiparasitism in this remarkable phase of hyperparasitism. This phenomenon could be explained by the continued abundance of these parasites in the locality, the non-availability of their preferred host species and the subsequent adaptability to the host available in plenty rather than to the laws of specificity and host selection. It is evident that when hymenopterous parasites exercise a wide and apparently indiscriminate range of host selection they will probably prove to be secondary rather than primary parasites.

Coppel (1951) has shown that the effectiveness of *Phytodictus fumeferanae* Rohw., a primary parasite of the spruce bud-worm *Choristoneura fumiferana*

TABLE I

The percentage and sequence of primary parasites on Hymenia recurvalis F.

Month	Number of Hymenia larvae collected from field and reared	Number of Apanteles adults			Number of Ichneumonids			Number of Braconids		
		Reared in the laboratory from larvae collected from the field	Pupae collected from the field	Percentage of parasitism	Reared from the host from larvae collected from the field	Pupae collected from the field	Percentage of parasitism	Reared in the laboratory from the field collected larvae	Pupae collected from the field	Percentage of parasitism
July	22	13	67	..	1	1	..	1	1	..
August	42	26	34	..	2	1	..	2	2	..
September ..	23	14	208	..	1	..	..	..	..	..
Total ..	87	53	309	61	4	2	4·6	3	3	3·4

TABLE II

The percentage of hyperparasitism on Apanteles new species and the degree of hyperparasitism among various hyperparasites and their seasonal occurrence

Month	Number of Apanteles pupae collected from the field	Number of pupae hyperparasitized	Total percentage hyperparasitized	Number of pupae of Apanteles hyperparasitized by						
				Elasmids	Cera-phronids	Miscogas-terids	Eurytomids	Eupelmids	Eulophids	Ptero-malids
July ..	67	42	..	22	15	3	2	Nil	Nil	Nil
August ..	34	25	..	6	5	3	7	4	Nil	Nil
September ..	208	130	..	62	Nil	4	Nil	Nil	57	7
Total ..	309	197	63·7	90	20	10	9	4	57	7

(After Narayanan, E. S., Subba Rao, B. R., and Ramachandra Rao, M. R. (1957), *Proc. Indian Acad. Sci.*, B **46**, 241-46)

(Clem.), was reduced to 17·9 per cent by hyperparasitism. Seven species of secondary parasites were involved in this.

When two or more secondary parasites occur in the same host, super- and multiparasitic relations are involved. This has been amply exemplified by Haviland (1922). The aphid parasite *Aphidius* may be attacked by the endoparasitic *Charips* or by the ectoparasitic *Lygocerus* or *Asaphes* or *Pachycreps*. The *Charips* along with its Aphidiud host perishes as the result of attack by one or the other of these ectoparasites. The latter on their part frequently suffer from the competition. Thus when two or three hyperparasites occur together irrespective of their belonging to the same or different species, only one survives or both may perish consequent upon the exhaustion of the available food. Parasites of a tertiary order are often referred to in literature, but there is not enough data to prove this occurrence. However, Muesebeck and Dohanian's studies (1927) on the chalcid *Pleurotropis* are noteworthy. Out of the several hundred cocoons from which the species *Pleurotropis tarsalis* Ashmead emerged, it was astonishing that ninety-seven per cent had developed at the expense of hymenopterous parasites. Only in very rare instances, the *Pleurotropis* itself behaved as a secondary parasite of the host concerned. Since *Pleurotropis* is an internal parasite which pupates within the larval or pupal covering of the host that it destroys, its actual biological relationship can be readily determined.

DISCUSSION

The biological control of insect and plant pests is a natural sequence to the occurrence of insect and plant parasitism in nature. Of the six principal methods of insect control, namely the mechanical, the cultural, the physical, the chemical, the biological and the legislative, the biological control of insect pests, sometimes described as the biological basis of insect control, has attracted much attention within recent years. As Munro (1931) observes: ' In the main the reasons for this are the astonishing successes of the late Dr. Muir in his Hawaiian work and the strong championship of parasites as controlling agents by Dr. Tillyard in Australia. The establishment at Farnham Royal of the Parasite Station of the Imperial Institute of Entomology has made control of noxious insects by parasites of special interest to the Empire, and the remarkable results recently obtained by Messrs. Tothill, Paine and Taylor in the control of the Levuana moth in Fiji by the tachinid parasite *Ptychomyia remota* have further added to the popularity of this method.' A much earlier and a more spectacular triumph than the Hawaiian introductions, a critical summary of which has been published by Imms (1926), was the introduction in 1899 of the Coccinellid beetle *Rodolia cardinalis* (Muls.) from Australia into California to subjugate the fluted or cottony cushion scale

Icerya purchasi Mask. Accidentally introduced into California from Australia or New Zealand through imported plants and bereft of the restraining influence of its natural enemies that kept the pest under check in its native habitat, the scale multiplied with such alarming rapidity that it threatened the orange and lemon groves of that state with extinction. Alfred Koebele of the United States Department of Agriculture was sent to Australia to find out if possible any natural enemy of the pest in its native home. As Howard (1930) puts it ' the results of Koebele's work are now known everywhere. The story has become a classic in Applied Entomology and Horticulture '. The small lady-bird beetle *Rodolia cardinalis* was introduced and the Coccinellid silently but effectively brought about such an appreciable reduction of the pest that it was no longer a menace.

Though these early successes created a vogue in parasite control, yet there are other reasons as well why the biological basis of insect control has been recognized by entomologists all over the world as an important weapon at the disposal of the economic entomologist. Mechanical and chemical control such as spraying, fumigation, etc., are either unsuitable in certain localities or are not always an economic proposition. Moreover, they have to be repeated year after year. Further as Thompson (1930) observed, ' they do not bring the population level of the pest low enough to prevent a recurrence or even an increase of damage in the following year. In certain parts of the world the amount of arsenic now used, during the ordinary treatment against the codling moth in apples, is so great that the arsenical residue on the fruit at the time that it is gathered is dangerously large and has to be removed by special processes. There is some reason to suppose that the arsenical compounds washed down into the soil may, after a certain length of time, prove injurious to vegetation. Furthermore, in some cases at least, there is definite evidence that certain species of injurious insects are becoming immune to some of the poisonous substances ordinarily used, such as hydrocyanic acid gas and lime sulphur wash '. There are also technical difficulties such as the availability at the required time and place of a team of personnel trained in the science and practice of using lethal insecticides. In cases like these the biological method of control offers a very promising and economical method of attack. But the introduction and establishment of parasites from one country into another is no easy matter. Munro's (1929) observations on this aspect of the problem has already been stated earlier. In many countries failures have been more frequent than successes and these failures and successes have been dispassionately discussed country by country by Howard (1930), Sweetman (1936) and Clausen (1956). The limitations of biological control in forest entomology have been discussed by Munro (1931) who gives a telling illustration of the inapplicability of the use of parasites to the control of wood wasp of the family Siricidae. These failures and limitations have only served

to emphasize the complexity of parasite introduction and workers from different countries of the world have made important contributions to the diverse aspects of parasitic study such as superparasitism, multiparasitism, hyperparasitism, specificity, power of prolificacy, etc. In this paper, the phenomena of superparasitism, multiparasitism and hyperparasitism are discussed in detail.

In a dispassionate discussion about superparasitism and its effect on the successful introduction of parasites from one country into another, one must take into consideration whether the phenomenon of superparasitism, as it occurs in nature, is desirable or undesirable. In those cases of superparasitism where all the individuals develop in harmony, where there is plentiful food supply and the effect of superparasitism is not observed either in the sex ratio or in the size or in the vigour of the parasites, there can be little doubt that superparasitism can be considered as a blessing, as the parasite is not dependent for the build up of its population on a large host population. Superparasitism of this nature is obligatory. In cases of superparasitism where parasite progeny are not able to develop properly into adults on account of the infliction of injury by the earlier hatched larva on its younger ones or by the release of some toxin by the one that hatches out thereafter and inhibits their growth and development, the rate of reproduction is impaired and the build up of the population seriously affected. In instances of this kind superparasitism is certainly harmful. Indeed, this aspect of superparasitism assumes particular significance in the laboratory mass multiplication of parasites where, in spite of all the precautions taken, superparasitism supervenes. In such cases, weak individuals emerge that are not active and sometimes do not even mate; several of these individuals have malformed genitalia and in many instances even ' runts ' are produced. This has serious applied importance. If such parasites are multiplied in large numbers in the laboratory and released in the field, the experiments are doomed to failure.

In conclusion it may be stated that the economic entomologist has to clearly distinguish these phases of superparasitism in which one is harmless, and indeed sometimes even beneficial, and the other directly harmful, being the causes of the production of weak individuals, high mortality, less percentage of females and the inability to mate successfully and in the production of a larger percentage of males that will bring about a staggering reduction in parasite population and ultimately make biological ventures total failures.

The phenomenon of multiparasitism is more complex than superparasitism. When two or more species of parasites compete in the same host the death of one or the other parasite oftentimes occurs because the specific fitness of one species makes it dominant over the other when they meet in competition in the medium of the host. Multiparasitism can be described as a tragedy and

a waste. Even the parasite that wins the battle is tired, weak and sometimes dwarfed. These effects are observed in its power of fecundity. Here is an example of a struggle for survival where the victor is none the better. For this reason multiparasitism can only be regarded as harmful. From the point of view of applied importance multiparasitism may have far-reaching reper- cussions in the introduction and establishment of parasites into a new country. If a parasite that is prone to multiparasitism is introduced it will neutralize the silent beneficial work of a useful parasite in the country of introduction.

Yet, in spite of what has been stated above, there is not enough data to prove that multiparasitism is unquestionably a harmful phase of insect parasitism. It is apparently true that the more prolific parasite that emerges as the victor may reach the high population level more quickly than the competitor that it destroys, and the destruction of the more prolific parasite by one of less prolificacy through multiparasitism should theoretically delay the time or period required for stabilizing the host parasite population. How- ever, when once the stability has been attained, the factor of prolificacy must practically be disregarded, since only a sufficient number of progeny mature to replace the parents and there is no increase. In this context the observation of Smith (1929) could be quoted: 'The case for or against multiple parasitism is, of course, not proven, and will not be until extensive experiments under controlled conditions have been carried out. Such experiments are now under way, but they can only indicate the principles under which multiple parasitism operates. Because of the complexity of a parasite's environment, it is doubtful whether one could determine, from studies other than actual field observations, just how it would react in the open and then it would be too late to make use of the knowledge gained '.

Probably, very few primary parasites of any given insect are free from being parasitized by other parasites which are technically termed hyper- parasites or secondary parasites. Consequently, hyperparasitism is a signi- ficant factor in the maintenance of insect population in nature. As Imms (1937) observes, 'the biological association of hosts, primary parasites, secondary parasites and tertiary parasites, if at all they are proved, is a highly adjusted complex '. From an evolution point of view hyperparasitism is a develop- ment of multiparasitism and, in some cases, it is truly difficult to distinguish between the two phenomena, since one may integrate into the other. The occurrence of hyperparasitism in any biological control venture can only be considered as destructive. A primary parasite imported for the purpose of introduction and establishment in the control of a pest may be overwhelmed by hyperparasites before it can succeed in establishing itself in the region. Unlike multiparasitism there are no two views on hyperparasitism, for and against, except in very rare instances like lac insect and silk-worm whose primary parasites are attacked by hyperparasites.

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RECENT PHYSIOLOGICAL AND CYTOLOGICAL INVESTIGATIONS ON PLANTS WITH MOTILE ORGANS

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Abstract

In the present review an account is given of some recent works carried out in physiological and cytological investigation on plants with motile organs in continuation of J. C. Bose's investigations.

As is known J. C. Bose chose two groups of plants with motile organs : (*A*) Plants with autonomous pulsating organs, of which the most striking example is the small leaflets of the Indian telegraph plant *Desmodium gyrans* and (*B*) Plants with anisotropic pulvini which are capable of nastic movement under variation of environmental conditions or under artificial stimulation. A short summary of these investigations as well as a list of Bose's physical and plant physiological monographs is given in the Introductory chapter of the Jagadish Chandra Bose Centenary Volume of the *Transactions of the Bose Institute*.

A. Plants with Autonomous Pulsating Organs

A.1. *Autonomous pulsation in* D. gyrans.—The plant on which the largest number of investigations have been carried out is the well-known leguminous plant *Desmodium gyrans*. We shall also describe similar investigations which have been carried out with another plant *Oxalis repens*.

The leaf system of *D. gyrans* consists of a petiole to which three leaves are attached, one large and two side leaflets. During the rains the young side leaflets of vigorous plants execute autonomous pulsations. A single detached leaflet with the cut end of its petiole dipped in tap water continues its autonomous pulsations, sometimes for a couple of days. Such an isolated Desmodium leaflet unit has many characteristics of an isolated animal (frog) heart kept in Ringer's solution. In both, the pulsations are myogenic only slightly affected by external stimuli like electric shocks; Bose (1928) found that they are both dependent on a supply of oxygen, similarly affected by temperature variations, by stimulants, depressants and poisons. One of his later investigations was on a comparative study on the effect of several Indian drugs on animal and plant contractile organs.

He had used a highly sensitive long period galvanometer to follow the variation of electric action potential accompanying the mechanical pulsation

of the Desmodium leaflet. The determination, due to the long period of the galvanometer used (10 to 12 seconds), could not follow accurately the electric variations in the petiole leaflet unit.

We give below a short account of recent investigations carried out in the Bose Institute on—

(i) the source of energy of mechanical energy of pulsation in *Desmodium gyrans* (1911–16) and *Oxalis repens* (1949-50) leaflet ; ·

(ii) more detailed studies on the electric variations accompanying mechanical pulsations (1957-58, 1958-59).

Autonomous pulsations in D. gyrans.—Bose in his studies on response to stimulation had made the assumption that the energy of such responses was derived from that of the stimuli. Depending upon the intensity of the latter. the response could be single or multiple. He tried to interpret the autonomous pulsations of a cut leaflet of *D. gyrans* on the assumption that in this case the energy of stimulation in the leaflet was stored up in a latent form— he however did not specify in what form the latent energy was stored. The investigation reported below was undertaken to elucidate this problem.

Usually the pulsations of the leaflets of *D. gyrans* are recorded on smoked glass plate recorder. For long period recording, the smoked glass plate was replaced by a photographic film, on which movement of a spot of light from a small mirror, whose axis of rotation is connected by a fine cocoon thread to the leaflet, recorded its pulsations. Usually records for 24 hours were taken.

Some interesting differences were noticed in the duration of the leaf pulsation depending upon the age of the leaflet.

A.2. *Pulsation in young Desmodium leaflets.*—The arrangement was placed in a south facing corridor so that the leaflet was illuminated by diffuse sunlight during the day. In Plate XVII four records are reproduced, of which *A* and *B* are records of leaflet pulsations when the cut end is dipped into tap water. From records made of diurnal variations in light intensity and of temperature, it appeared that the stoppage of pulsation at night coincided mainly with the dark period. In one experiment a young leaflet ceased to pulsate for three consecutive days due to persistent cloudiness of the sky. When the sky cleared up the leaflet started pulsating again. As a working hypothesis it was assumed that the energy required for the mechanical pulsation was supplied by the breakdown of the carbohydrate manufactured in the leaflet due to daytime photosynthesis. To test this point, the cut end of the leaflet was kept dipped in one per cent glucose solution and as before placed in diffused sunlight of the corridor. Curves *C* and *D* show that under such condition the pulsation continued nearly throughout day and night, showing that the leaflet movement could be maintained by
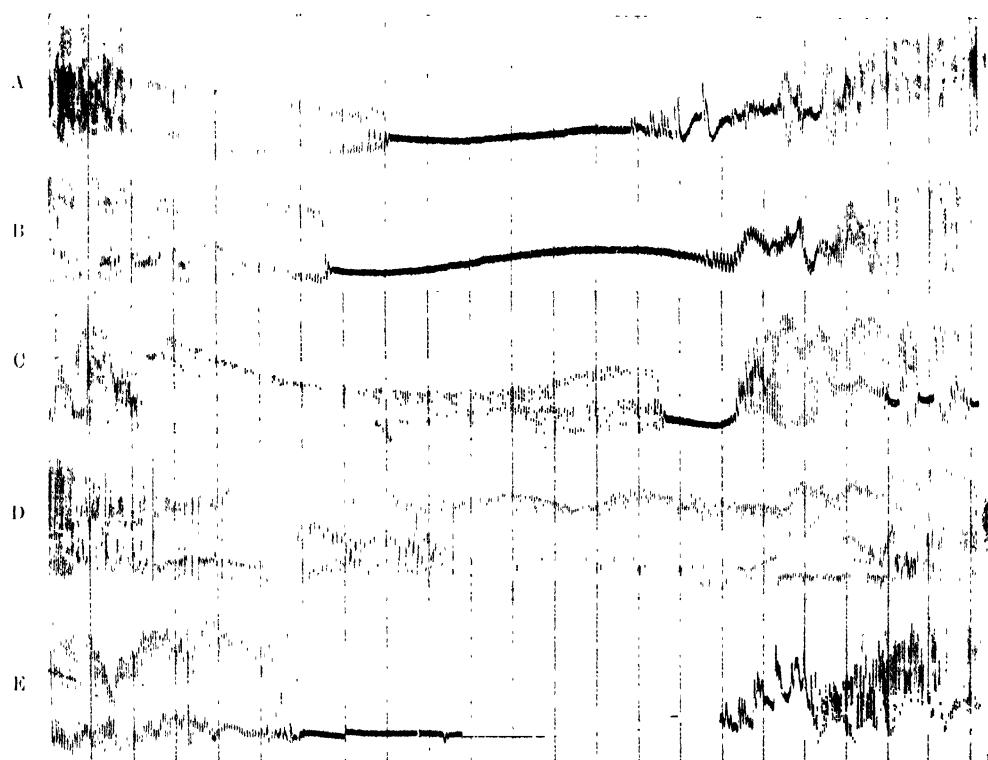
FIG. 1.   Serial diurnal records of an excised young leaf of Desmodium showing the effect of
              glucose on its pulsatory activity.
          A and B, normal records; note stoppage of pulsation at night and its revival at day-
              break; C and D, effect of 1 per cent glucose, showing disappearance of nocturnal
              stoppage; E, shows return to normal condition after withdrawal of glucose.
          Recording started daily from 1 p.m.   Black vertical lines indicate hour-marking.

the dismutation of the carbohydrate either photosynthesized in the leaflet during the day or during the night by the breakdown of the glucose absorbed from the solution. With several specimens of young leaflets the pulsations could be maintained during the day for over 20 days if the leaflet is kept exposed to diffuse daylight; if kept supplied with one per cent glucose solution in addition it pulsates continuously day and night during this period. During this time the colour of the leaflet changed from yellow-green to dark-green, showing that chlorophyll synthesis had taken place in the leaflet under such condition. If, however, the leaf is kept in complete darkness and at the same time the cut end is supplied with one per cent glucose, the pulsations continued in comparatively feeble manner till the second day of its treatment, after which the pulsations stopped and did not resume when the leaflet was again brought to light. It shows that daylight has some influence on the leaflet metabolism other than photosynthesis.

The difference in pulsatory activity between the young and mature leaflets is interesting. The young leaflets stop pulsating at night and resume it again at daybreak. In the mature leaflet which has a deeper green colour the pulsation could not be started again after it had stopped once, as in the young leaflet. The mature leaflet continued pulsation for two days and nights before coming to a stop. This can be attributed to the mature leaves' greater power under normal conditions of manufacturing carbohydrate, so that its night pulsation could be maintained from its surplus stored carbohydrate. But why the detached mature leaf could not resume its pulsation under diffused daylight condition once its continuous pulsation ceases, is not clear.

The leaflet of *Oxalis repens* also exhibits a rhythmic pulsatory movement though the frequency is much less in comparison to that of Desmodium. A mature leaf executes 8 to 9 continuous pulses in 24-hour time period. In this leaf also in the young stage pulsation remains stopped at night. But when supplied with glucose the young leaf pulsates continuously without nocturnal stoppage (Fig. 2).

A.3. *Respiration of the pulsating leaflet of Desmodium.*—It has been shown in the previous section that some form of carbohydrate, whether manufactured in the leaflet of *D. gyrans* by photosynthesis or absorbed through its cut stem from a dilute glucose solution, is utilized for the maintenance of its mechanical pulsations. In animal tissues muscular activities derived from breakdown of stored glycogen or fat are accompanied by rhythmic respiration.

The following investigation was undertaken to determine the respiration of the Desmodium leaflet due to its mechanical pulsation.

The apparatus employed is essentially a microrespirometer, consisting of two small stoppered glass vessels *A* and *B* of equal volume joined by a
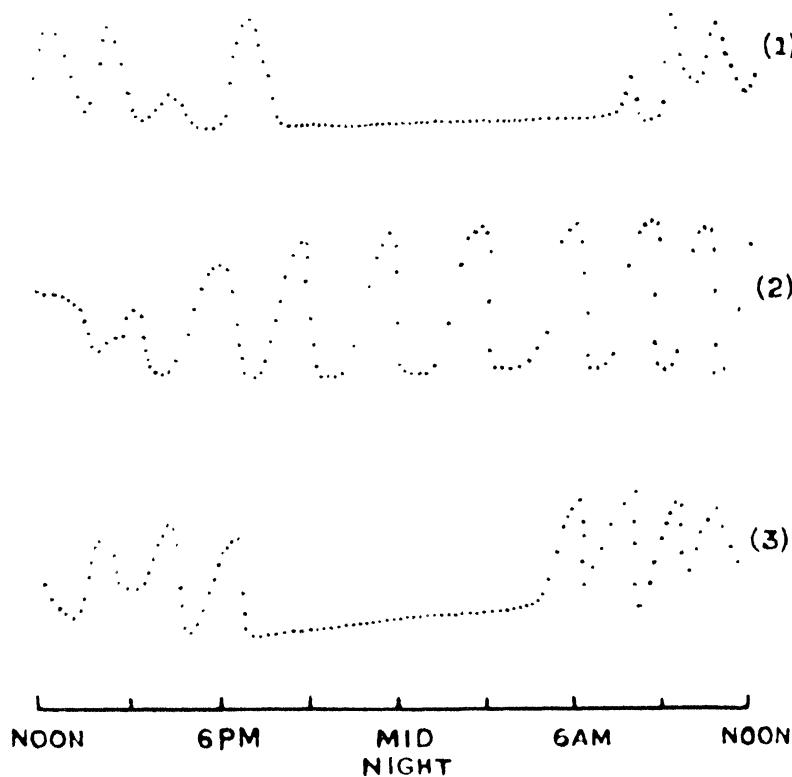
FIG. 2.   An effect of application of 1 per cent glucose on the motility of young leaflet of *Oxalis repens*.   (1), normal record showing motility during day and quiescence at night; (2), continuous motility during day and night after application of glucose; (3), return to normalcy after withdrawal of glucose.

capillary tube *C*.   In a small side tube attached to *A*, a leaflet of *D. gyrans* with its cut end wrapped in moistened cotton wool is kept.   At the bottom of the vessel *A*, blotting papers moistened with caustic soda solution are kept.   The CO_2 respired by the leaflet is absorbed in the latter.   The consequent diminution of the air volume in *A* causes a movement, towards *A*, of a small drop of light oil in the capillary tube separating *A* and *B*.   The respirometer is then immersed in a rectangular vessel with plate glass sides, filled with water.

With two telemicroscopes, the vertical pulsations of the leaflet tip and the horizontal motion of the oil drop meniscus are followed by two observers. The displacement of the two telemicroscopes after suitable magnification

by levers are recorded one above the other on a horizontally moving smoked glass plate.

In Fig. 3 is reproduced simultaneous record of the mechanical pulsation and respiration of a Desmodium leaflet. The first curve has a horizontal base line and the second an inclined one, giving the average rate at which CO_2 is exhaled from the leaflet and absorbed in the blotting paper moistened with caustic soda. The number of peaks in curve P is 14 and in R is between 13 and 14, i.e. they both have the same periodicity with a half period difference in phase. The up movement in the respiration curve coincides in period with the down movement of the pulsation curve, which represents actually an up movement of the leaf; it represents the inhalation of air by the leaflet during its up movement. The down movement of the respiration curve represents the exhalation of the inhaled air; the vertical difference between each up and down stroke represents the volume of CO_2 respired by the leaflet during each pulsation which becomes absorbed in the caustic soda soaked blotting paper.
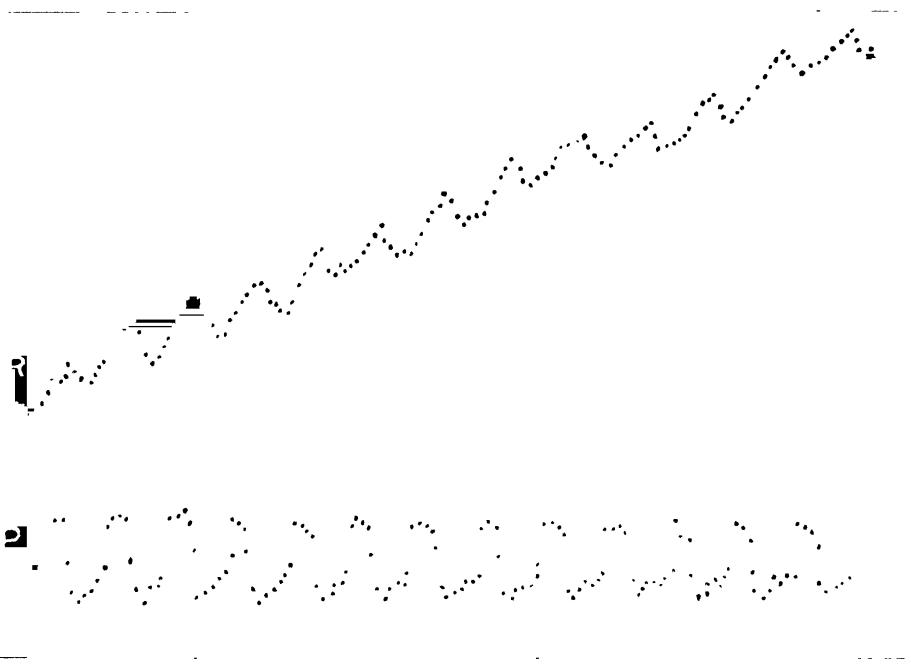
FIG. 3. Normal records of respiration and pulsation of a single Desmodium leaflet. R, rate of respiration; P, pulsation. Note pulsatory periodicities in the rate of respiration similar to pulsation curve.

A.3.1. *Further studies with the microrespirometer.*—It has been mentioned earlier that a one per cent glucose solution, introduced at the cut end

of a Desmodium leaflet, helped to restore the depleted store of carbohydrate produced in the leaflet during daylight photosynthesis. A series of investigations was undertaken with the respirometer to study the effect of introducing by turns small quantities of glucose, its breakdown products, and some C_4 compounds, on the respiration and pulsation of leaflet specimens. Some of the results obtained are summarized below:

   (i) Treatment with one per cent glucose produced a nearly 200 per cent increase in respiration with no significant alternation in the frequency or amplitude of pulsations.

  (ii) Treatment with glucose-1-phosphate (breakdown product of starch) and pyruvate (breakdown product of all carbohydrates) did not induce any increase in respiration. With continuous treatment, these substances produced toxic effect.

 (iii) Succinate, citrate and malate by themselves produced no marked increase in respiration: the continued application in solution produced no toxic effect.

 (iv) Only in case of the malate was it found that a combination of 0·05 per cent malate with 0·0005 per cent indole acetic acid (IAA) increased the rate of respiration by about 52 per cent while the same concentration of IAA applied singly produced a 13 per cent increase in respiration.

Further biochemical studies with these organic solutions tagged with radioactive isotope will be undertaken to find out whether the negative results in all instances except with glucose and malate were due to selective permeability of the cell membrane for these two compounds only. Other interesting problems which require to be followed up are: what is the fraction of the glucose absorbed in the leaflet and is exhaled as additional CO_2 and what happens to the balance of glucose absorbed.

A.4. *Electric pulsations accompanying the mechanical pulsations in the leaflet unit of* D. gyrans.—The relation between the mechanical pulsation and the electrical activity of the animal heart has been the object of a long series of investigations, ever since the string galvanometer constructed by Einthoven enabled him to obtain electrocardiogram of the heart. The relation between the two and which of them is the precursor has been a constant object of discussion amongst heart physiologists.

Bose was the first to obtain photographs of electric pulsations with a long period sensitive galvanometer. He found that as in animal heart such pulsations in *D. gyrans* were associated with electronegativity and the electric pulsations were almost replicas of mechanized pulsations. Recently a multichannel electric pen recorder capable of responding up to frequencies of 10 kilocycle per sec. has been used, to record the electric pulsation of the leaflet;

the mechanical pulsation of the leaflet was recorded on a synchronized smoked plate (Fig. 4). Below is given the electrocardiogram of a human heart. It will be noticed that similar to what has been found in the case of the animal heart (i) there is considerable deviation between the mechanical and electrical records, (ii) some similarity in the electric recording of pulsations in animal heart and *D. gyrans* leaflet.
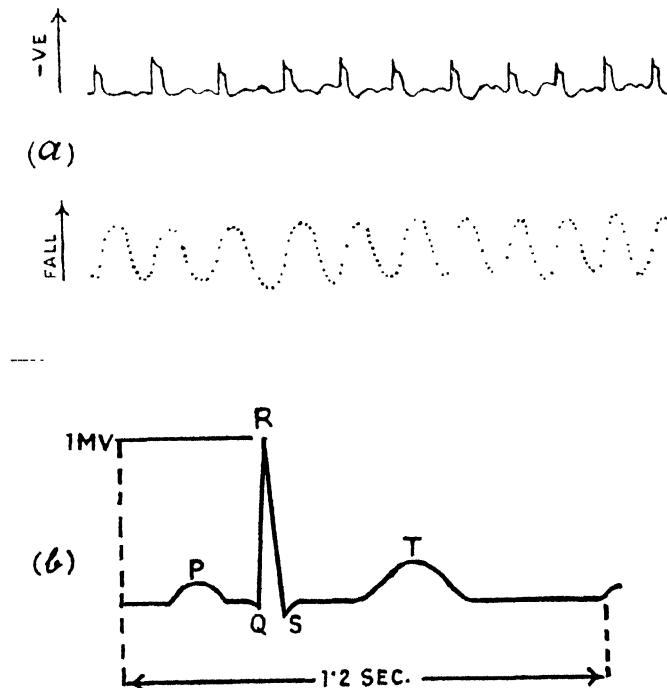

Fig. 4. Simultaneous record of the mechanical pulsation of Desmodium and of its electrical correlates.

(*a*) upper, electrical; lower, mechanical.

Compare the single electric pulses of Desmodium with the normal heart signal of man, shown in (*b*).

In the latter there is a sharp spike corresponding to the *R* spike in the electrocardiogram. With an electric recorder of higher amplification it will be possible to study in more detail the electric pulsation in *D. gyrans* leaflets. However, even with the present arrangement it is possible to undertake an experiment with *D. gyrans* leaflets which is not possible with an animal heart, viz. to find out what happens to the electric pulsations when the mechanical pulsations are forcefully stopped. It has been shown in some of our records that under such conditions the electric pulsations continue

unabated. It shows that the electric pulsation is intimately connected with the biochemical change which follows the process of stimulation of which the mechanical changes are secondary effects.

B. PLANTS WITH ANISOTROPIC MOTILE ORGANS

The second group of motile plants investigated by Bose responds to artificial or environmental stimulation by nastic movement of its anisotropic pulvinus. *Mimosa pudica* is a typical representative of this group. Bose discovered that when a stimulus is given at any point on the stem or on the leaf system, the resulting excitation is conducted along the stem petiole leaf system as an electrical action potential which could be detected by electric instruments; this excitation when it passes across the contractile main pulvinus or subpulvini give rise to mechanical response in the form of fall of main pulvinus, closure of the leaf system, etc. In fact a petiole pulvinus unit of *M. pudica* has some characteristics common with a frog nerve muscle unit. This conclusion of Bose is based upon the following observations on changes in the magnitude of mechanical response: (i) diminution under a physiological block produced by cooling with iced water the connecting petiole, (ii) polar action of current, viz. diminution respectively, enhancement of response depending on the direction flow of a steady current through the petiole, (iii) the enhancement of response by stimulants like alcohol, its depression and eventual abolition by the action of poisons like copper sulphate, chloroform, etc.

By means of an electrical probe first devised by himself Bose discovered a particular layer in the petiole which preferentially carried the electric excitation. In addition to Bose's theory of conduction along specialized tissues in plants by means of action current, there are two other theories of transmission of excitation in *M. pudica*: the hydromechanical theory of Haberlandt (1914) and others, and the chemical mediator theory first put forward by Ricca in 1916. These will be discussed in a following section (B.2). In the present review we shall report on the continuation of some aspects of Bose's investigation under the following heads:—

(1) Active substance present in the pulvinar cells of *M. pudica* and allied plants.

(2) Chemical mediator of excitation.

(3) Sap exudation from contractile pulvinus following stimulation.

(4) Autogenic movement of pulvinus petiole unit.

(5) Electric correlate of transmission of excitation in *M. pudica*.

B.1. *Active substance.*—Bose (1928) described the presence of such a substance in the pulvinar cells of the motile plants of the Mimosa and other species. They are stainable by safranin, haematoxylin and some other vital

stains. He found a parallel between the density of stainable substance in the pulvinar cells and the motility of the plant organ. His attention had been drawn to the observation of an Austrian physiologist to the presence of a highly stainable substance in the interstitial granules of breast and wing muscles of birds like falcon, geese and fowl. The density of this stainable substance in the bird tissue was found proportional to its muscular activity. Bose concluded that the stainable substance present in the contractile organs of plants and animals are of a similar nature, and named it the *active substance*. Further chemical tests carried out on these plant tissues showed that this stainable substance had unsaturated carbon bonds, that it was neither a fat nor a lipid substance.

At the time (1944) when investigations on the nature of the active substance was again taken up in the Bose Institute, attention had been drawn to the investigations of Kuhn and Moewus (1938) on the role of some carotene compounds like crocin and crocetin in producing the motility of the flagella of the alga *Chlamydomonas eugamatos*, and also in determining its sex character. It was considered worth while investigating whether the unsaturated compound believed by Bose to be the 'active substance' present in the motile plant organs was a carotene compound. The first step in this investigation by Banerjee *et al.* (1946) was the preparation of crocin, crocetin and other carotene compounds from saffron. The next step was the detection of such compounds in *M. pudica* and other motile plant organs.

For this purpose Molisch's test solution II, a solution of paradimethyl-amino-benzaldehyde ($C_6H_6H(CH_3)_2CHO$) in sulphuric acid, was applied to a pulvinar section. According to Molisch a deep crimson stain forms indicating the presence of a phloroglucinol tannoid substance in the pulvinus. In the present instance not only this colour was formed but in the liquid, orange coloured crystals were found which were identical under the microscope with transcrocetin dimethyl ester, a substance which had previously been obtained from Indian saffron by B. Banerjee. This crystal indicated the presence of crocin or allied diglucoside of crocetin in the pulvinar region. Such glucosides were found to be present in other motile pulvini in concentrations proportional to their motility. They were believed to be the 'active substance' of Bose.

The carotenoid compounds found in the motile plant organs however did not possess the stainable character of the active substance found by Bose. Later investigations by research workers in Prof. Green's laboratory in Wisconsin have shown (1953 Director's Report) that the interstitial granule, present not only in the active muscle but also in actively respiring organs like liver and kidney of many birds and small animals, could be identified with mitochondria like bodies, which are particulate bodies containing related enzymes taking part in the Kreb's cycle.

B.1.1. *Cytological studies.*—The presence of mitochondria like bodies were now sought for in the plant organs. In the pulvinus of *M. pudica* (Dutt 1954-55) several mitochondria like bodies revealed their presence in small numbers but these were only stainable with Janus green and not with safranin and similar dyes, so they could not be the active substance of Bose. Further studies were carried out by Dutt (1955-56) on the behaviour of the mitochondria of the motor cells in comparison with those found in cells of non-motile plants. It was also noticed that the complex chloroplastid bodies, found in all green plants and in the Mimosa leaf blade, are totally absent in the pulvinus. This total absence is correlated with the fact of the absence of starch grains in the pulvinus, where the carbohydrate occurs probably in the form of sugar. It was however observed that in the dark the mitochondria coalesce together to form plastid like bodies.

In course of the search for Bose's active substance as described above, the conclusion reached was that this substance could not be identified either with crocetin or with mitochondria. Mridula Dutt (Annual Report, page 9, 1954-55) then took up a systematic cytological study of the active pulvinar cells of the group of plants with motile organs. She started from the observation of Molisch that, using what is known as his solution II, the pulvinar section took a deep stain, due to the presence of what Molisch called phlorogluco tannoid bodies. The presence of such tannin vacuoles in pulvinar cells had also been observed by Guillermond (1941), Weintraub (1952) and others.

The plants investigated by her belonged to two groups: (*a*) which showed nastic movement on stimulation, plants like *M. pudica, M. speggazzini, Biophytum sensitivum, Averrhoa carrambola.* Of these *M. pudica* has been most intensively studied, (*b*) those which exhibit autonomous pulsations like *Desmodium gyrans* and *Oxalis repens.*

The active contractile cells in these plants contain pronounced vacuoles which are specially large in *M. pudica.* The cells are stained not only by safranin, neutral red, etc., but by specifically tannin staining compound like Molisch's reagent II, reduction of osmic acid, etc. The contractile vacuoles in the pulsating pulvini of *Desmodium gyrans* have not yet been studied in detail.

The general picture obtained from such cytological studies made by Mridula Dutt is as follows (Dutt 1955-56).

The pulvinar cell is contained in an elastic wall with a thin lining of cytoplasm. Within this cytoplasm are included particulate bodies like a large nucleus, mitochondria bodies and a large complex vacuole. There are no chloroplasts enclosing starch grains. The vacuole, enclosed in semipermeable membrane, has in addition to its sap contents also a body made up of a complex of tannin with probably protein like body. These conclusions are based on the following observation:

The small depth of cytoplasm becomes visible, when the active vacuole was stained with brilliant cresyl blue; one part of the vacuole enclosure showed up as violet in colour, characteristic of the alkaline salt containing sap and the other portion the acid phenolic (tannin like) component stained blue. It was found that when the tannin component was removed by tannin solvents a residual substance remains—which gave positive reactions with several protein tests. This tannin body is always placed centripetally in the vacuole. The vacuole in the expanded state contains several salts in solution of which potassium salts are important. As a result of the stimulation the vacuoles along with the enclosing cell collapse liberating the salt solution. The tannin body however retains its shape, and does not perceptively decrease in size after stimulation.

The above picture of the contractile cell is not exhaustive.

The complete picture of its structure, of its constituents with their interrelations should provide all the data necessary to explain the contraction and recovery of the contractile cell from stimulation. One remarkable fact which has been observed by different investigators is (i) the presence of tannin body in the vacuole which forms a complex with a protein like substance, and (ii) that the concentration of the tannin body appears to increase with the motility of the cell. There are two protein linings forming semi-permeable membranes, (i) enclosing the cytoplasm and (ii) the vacuoles. Why the activity of these contractile semi-permeable membranes should depend on the tannin concentration is not yet understood. The presence of tannins has been reported in other contractile plant organs, like the guard cells of leaf stomata, the motile organs in the carnivorous plant Drossera.

B.2. *Chemical mediator in transmission of excitation in* M. pudica.—After discussing the available knowledge on the structure and the constituents of the contractile pulvinar cells, we pass on to consider the process of stimulation of such cells. In the usual experiments a point on the stem, the pulvinus, or the leaf system is stimulated, by local heating, electric shock, mechanical blow, etc.; the resulting change whether local or transmitted is recorded; the mechanical response takes the form of fall of pulvinus, or collapse of a leaf system. Sometimes it is possible to measure the electric action potential which accompanies the transmission of the excitation from the point of application of stimulation to the point of detection. The velocity of transmission can be measured by noting the time interval between stimulation and the mechanical response and also in certain cases by noting the time of passage of the electric action potential across two points on the sensitive plant.

B.2.1. *Velocity of transmission.*—The velocity of transmission of excitation and its accompanying action potential is not constant but depends (i) on the age of the plant organ on which the measurement is made, (ii) on the intensity of stimulus, and (iii) time of the year.

As will be discussed later, the velocity of transmission of excitation can be roughly divided into three groups:

(1) Fast—velocity from 180 to 300 mm./sec. found usually in young leaf system and also resulting from strong stimulation. Such velocities can only be determined from mechanical response. It has not yet been possible to electrically measure this fast velocity of transmission.

(2) Medium—velocity between 16 and 110 mm./sec. Its velocity of propagation can be measured from observation based on both mechanical and electrical methods. This is the region of propagation due to physiological variations, which has been studied in detail by Bose.

(3) Slow—Ricca in 1916 took a shoot of *Mimosa speggazzini*, cut it into two; they are then connected through a glass tube filled with water. An intense stimulation of the lower limit by a flame is often followed by a fall of leaf in the upper limb. Ricca's observation, which has been confirmed on *M. pudica* by Snow (1924) and others, shows that it is possible to collect from the cut end of a stimulated motile plant organ sap which when introduced to a leaf pulvinus unit of *M. pudica* will produce closure of the leaf system. In some cases it is possible to observe multiple response with several closures and reopening of the leaf system taking place under such condition. Thus it appears that during stimulation of a sensitive plant organ, a sap is secreted, which when introduced into the cut end of another leaf system will reproduce the response usually produced by direct stimulation in an intact plant. The velocity of propagation of the slow excitation has been found to vary between 2·5 and 5·0 mm./sec. while the maximum speed of ascent of sap in *M. pudica* has been found to be 3·00 mm./sec. To use the term which has been coined to describe the behaviour in an animal nerve muscle system, in which acetyl choline secreted during nerve stimulation at the nerve muscle junction can reproduce, when injected in dilute solution into the veins of a denervated muscle twitching, the irritability substance in *M. pudica* can be described similar to acetyl choline as neuromimetic.

After Ricca's observation had been confirmed, a number of investigators including Umrath (1930), Fitting (1936), and Hesse (1939) have tried to isolate this chemical mediator or, as we shall denote, the irritability substance (I.S.) in as pure a form as possible and to determine its chemical structure. Since the I.S. is carried along with the transpiration current, it must therefore be

water soluble.   Its method of isolation first worked out by Umrath is based upon preliminary investigations that it is a weak nitrogen containing oxyacid.

B.2.2.   *Irritability substance*.—The usual method used for isolating the I.S., which preliminary investigations had shown to be a weak oxyacid containing nitrogen, is due to Umrath and Soltys, Fitting and Hesse (D. M. Bose 1949–51).   In this method the crushed leaves are extracted with boiling water; after concentration, the solution is precipitated with 90 per cent alcohol, which brings down the protein and other bodies.   The solution left is then precipitated by basic lead acetate; the precipitate is decomposed with SH_2.   The solution is further purified by precipitation with mercuric acetate, which is again treated with SH_2.   A highly purified concentration of I.S. is attained, which decays with time, showing that it is an oxidizable substance.

Hesse using a slightly different technique has obtained an amorphous concentrate which under favourable condition gives the Mimosa test (viz. the highest dilution which will induce response when introduced into the cut end of a Mimosa leaflet) at a dilution of $1 : 5 \cdot 10^8$.   The substance behaves as an oxyamino acid and has a molecular weight between 300 and 450.   This line of investigation was taken up by Banerjee *et al.* (1944–46).   It was not possible, partly due to technical inadequacy, to confirm Hesse's final findings.   At the same time it may be noted that Hesse's white amorphous substance and its molecular constitution has not been independently determined.

After the chemical department of the Institute had become well equipped with different chromatographic separation methods, the attempt at isolating the I.S. from *M. pudica* was resumed by Chakrabarty (1954-55).   To the aqueous pulvinar extract from Mimosa mercuric acetate was added.   I.S. was precipitated as a mercury compound whose decomposition with SH_2 gave an aqueous concentrate showing pronounced Mimosa (chemonastic) effect. The solution gave positive ferric (phenolic) and ninhydrin (amino acid) reactions.   When subject to paper chromatographic analysis, three ninhydrin sensitive spots were obtained, which were identified as due to glycine, alanine and glutamic acid.   For better purification, in another investigation, the plant extract was treated with copper acetate.   The precipitate formed after decomposition with SH_2 gave a precipitate which did not give the Mimosa test but the ferric chloride reaction, showing that the phenolic compound had been removed.   The soluble compound after being freed from copper in the usual manner showed greater Mimosa test activity.   By chromatographic test again the three amino acids, viz. glycine, alanine and glutamic acid, were detected.

It is interesting to recall that in 1930 Fitting had tested a large number of water soluble organic compounds commonly occurring in plants for their chemonastic (Mimosa test) properties.   He concluded that glycine, serine,

alanine and glutamic acid are the most active; but neither he nor other later workers could specifically demonstrate their presence in *M. pudica* extracts.

The present investigation supplies definite information about their occurrence in the water soluble extract from *M. pudica*. In the following year (1955-56) the sap obtained *directly* from stimulated *M. pudica* leaflet was subjected to chromatographic analysis with water saturated phenol as the solvent. Again three spots corresponding to glycine, alanine and glutamic acid were obtained. Of these three amino acids the glutamic acid is the most active as irritant for the Mimosa effect.

Chakrabarty (1956-57) examined chromatographically the content of sap extracted from stimulated leaflets of other pulvinar active plants. Only in *M. speggazzini* was the presence of two amino acid spots noted. At the same time the seasonal variation of the amino acid content of this sap from *M. pudica* was studied from September to March of the following year. During this time of the year, the reactivity of the pulvinus to stimulation is sluggish. It was found that only during September did the sap disclose the presence of all the three amino acids; while from November to March only two amino acid spots corresponding to glycine and alanine were observed. That due to glutamic acid was missing. These investigations carried out in this Institute have shown that amongst the irritability substances secreted by different organs of the Mimosa and allied plants these three amino acids, glycine, alanine and glutamic acid, are present. But it cannot be said that these three amino acids, of which glutamic acid is the most important, alone constitute the irritability substance (I.S.).

It is interesting to note that glutamic acid (Vbra 1955) plays an important role during the degradation and resynthesis of brain tissues of exercised rate during fatigue and recovery from fatigue.

B.3. *Sap exudation from contractile pulvinus of* Mimosa pudica *following stimulation*.—In a previous section when considering the pulsatory activity of *D. gyrans* we had drawn an analogy between the autogenous rhythmic pulsation in the small leaflet of *D. gyrans* and that of an isolated animal heart. In one respect the analogy does not hold good. Each systolic contraction of the heart is accompanied by the expulsion of a fluid, the blood, which flows back to the heart during the diastole. In this section we shall consider the process of sap exudation from the cut ends of (i) a *Mimosa pudica* petiole unit under stimulation and (ii) of a similar unit of *D. gyrans* when pulsating spontaneously.

B.3.1. *Mimosa*.—From the observation of Pfeffer (1905) and Bose, it was generally accepted that the response movement of the pulvinus petiole unit of the *Mimosa pudica* is accompanied by loss of turgor due to expulsion of sap from the pulvinus. The qualitative observation of these pioneers has been followed up recently by a more quantitative study of the expulsion and re-absorption of sap following the fall of a *M. pudica* leaflet due to stimulation

and its subsequent recovery (1956-57). The apparatus employed is a micro-potometer. The specimen used in such investigation is a petiole pulvinar preparation with a stem piece attached to it. It has three cut surfaces, two at the upper and lower ends of the stem piece, and the third at the petiolar end. Two of the cut ends, viz. upper end of the stem and the cut petiole end, are sealed, and the third open end is connected to a capillary tube filled partly with water.

On stimulation two responses are observed : (i) the movement of sap exudation and its reabsorption at the open cut stem end and (ii) the fall and recovery of the petiole end. The former, recorded by a travelling microscope, is, after conversion through a lever arrangement, recorded on a horizontally travelling smoked glass plate. The mechanical movement of the sealed petiole, amplified by a simple lever arrangement, is also recorded on the same smoked glass plate. In Fig. 5 the upper curve recorded the outflow and inflow of sap and the lower one the fall and rise of the free petiole end.

The outflow of sap is represented by the up curve and the inflow by the down curve. The initial portion of the curve represents the residual suction rate; this was taken as the base line for computing the values of the suctional changes, due to responsive movement of the pulvinus. In the present record the volume of water expelled during contraction and reabsorbed during recovery has been found to be 0·0192 and 0·036 cu. mm. respectively. This difference between the quantities of expulsion and absorption indicates that the whole amount, dislodged from the pulvinus during its contraction, did not escape through the cut. end introduced into the potometer; a part escaped through the epidermal outlets. During recovery the epidermal outlets were presumably closed, the total deficit in the pulvinus had to be made up by suction through the vascular channel connected to the potometer; hence the volume imbibed through the potometer during recovery greatly exceeds the volume exuded during pulvinar contraction.

The ratios of outflow and inflow of water through the different cut ends introduced into the potometer were found to vary greatly. Flow through the upper cut end of the stem indicated lowest ratio and that through petiolar cut end highest. This showed that the water dislodged from the pulvinus encounters least resistance in its flow through the petiolar tissues. The presence of large intercellular spaces in the petiole, as reported by Toriyama (1955), may be the cause of low resistance of water transport in that direction.

Bose suggested that the extruded sap is translocated to other directions through the cortex. Findings of the present investigation prove that water dislodged from the pulvinus during excitatory contraction flows through channels where it gets least resistance. In the intact plant water, expelled from the contracted pulvinus, escapes through the epidermal outlets or it is
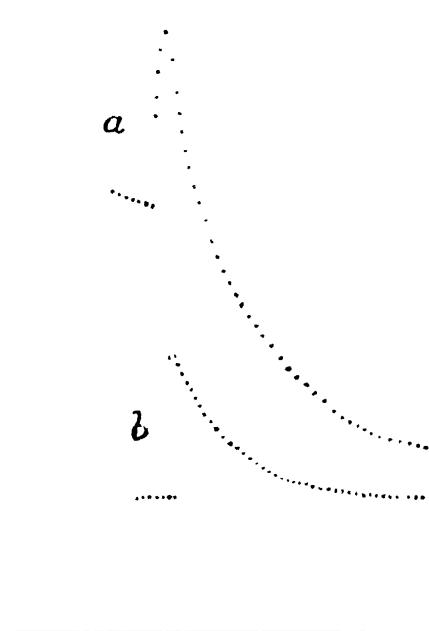
FIG. 5.   Record showing expulsion of sap from the pulvinus of Mimosa due to its contractile
response.
   Curve a, showing expulsion of sap by up curve and subsequent recovery of suction rate
      by down curve; note absorption of greater quantity of water during recovery.
   Curve b, showing contractile response and recovery by up and down movements respec-
      tively.
Dots at intervals of 24 secs.

retained in the adjoining tissues, if they are not fully turgid, to be reabsorbed
from there during recovery.

   B.3.2.   *Desmodium.*—In order to study whether the autonomous motility
of the pulvinus of *D. gyrans* also brings about a change in its turgidity, the
suctional rate of the leaflet of *Desmodium gyrans* was similarly studied with a
potometer with a much narrower cross-section.   The suctional activity and
the pulsatory movement of the leaflet recorded simultaneously is given in
Fig. 6.

   It will be noted that there is no change in the suction rate through the
potometer corresponding to the up and down movements of the leaflet.   A
couple of alternative interpretations is possible:

   (i) The volume changes of the Desmodium leaflet pulvinus petiole is small
compared to the sensitivity of the micropotometer.
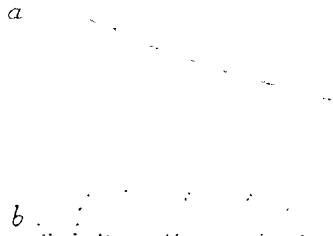
FIG. 6. Record showing no change in the suction rate of the leaflet of *Desmodium gyrans* during its autonomous movements; *a*, residual suction rate (down curve) in moist chamber; *b*, autonomous rhythmic pulsation.

Dots at intervals of 12 secs.

(ii) The autonomous movement of the Desmodium leaflet is not a simple linear up and down movement of the Mimosa pulvinus petiole unit. The path described by the petiole has been found in another experiment to be more of a gyratory nature, made up by the superposition of two linear motions at right angles to each other, with slightly incommensurable phase difference (1942-43). The motion is in some ways similar to the whiplike motion of bacterial flagella. Astbury and Weibull (1949) have shown that protein moiety of the flagella gives X-ray diagram similar to that of myosin. Manton (1953) has shown that the flagella is made up of about 9-11 strands and the successive phased contraction of these sets of fibres gives rise to the whiplike motion. Probably the gyratory movement of Desmodium leaflet is due to the contraction of several constituent fibre elements in succession. Under such condition, as in the contraction of myosin fibril in skeletal muscle, no sap exudation is to be expected. It will require X-ray diffraction pattern studies of the twisting petiole of Desmodium leaflet to find out whether myosin like fibrils take part in the pulsating movement of the twisting petiole. On the other hand there is a great deal of similarity between the contraction of the *Mimosa pudica* pulvini with animal organs enclosing cavities filled with some fluid, like the heart, the alimentary canal, the pancreas and probably the lungs. There is a very interesting similarity between the contractile vacuoles of pulvinar cells in *M. pudica* and the mechanism of expulsion of water from a contractile amoeba vacuole following stimulation, when a reduction of vacuolar volume occurs and certain openings are formed on the cell walls through which water is expelled during contraction. The time of recovery is much longer than that of contraction. The systole and diastole of the amoebic vacuole

contraction show a great deal of similarity with that of the *Mimosa pudica* pulvinus.

These animal vascular organs besides responding to stimulus can also undergo autogenous or spontaneous contraction. The following investigation shows that pulvinus petiole units of young Mimosa leaflet on occasion exhibit such autogenous movement.

B.4. *Autogenic activity of the pulvinus petiole units of M. pudica.*—It was observed by Bose that the leaves of Mimosa sometimes execute responsive movements, without any apparent excitation. He did not however make any detailed study on this behaviour of the plant. This phenomenon came to his notice during his daytime observations, and he concluded that such response is due to over-accumulation of energy during daytime in the highly sensitive condition of the leaf, which it releases in such responses in a semi-autonomic manner.

In recent experiments carried out in the Institute, diurnal records of the movement of some leaves showed that such responses can appear not only in daytime but also at night (1956–58). In making a thorough investigation of the problem it has been found that the leaf exhibits this behaviour in its young stage which gradually disappears with the attainment of maturity (Fig. 7).

It will be found from the record that the responses are randomly distributed throughout day and night. Such responses cannot, therefore, be due mainly to environmental factors.

It further shows that the performance of the responses is not dependent on higher excitability of the leaf as Bose had assumed. Bose's own experiments have shown that the tonic level of the leaf is raised higher in the daytime and it is more excitable during the daytime than at night. His experiments have further shown that the young leaves are less excitable than the mature ones.

This autogenic activity of the young leaf was found to disappear with the removal of the top bud just above it. And it was partially revived by application of indole acetic acid at the cut surface after removal of the bud.

Cessation of autogenic activity under detipped condition and its partial revival by application of auxin on the cut surface indicate that hormone mobilization from the apical bud has some relation with the activity. But how it is correlated with the activity is not clear. Moreover, the question arises that if the hormone is transported to other leaves down the stem, why it

Fig. 7.   Diurnal records of the movement of a young leaf of *Mimosa pudica* showing autogenic responses. Records of seven consecutive days starting from very young stage of the leaf are given in the curves 1, 2, 3, 4, 5, 6 and 7.

Note decrease in frequency of autogenic responses in the lower curves with advancement of maturity of the leaf.
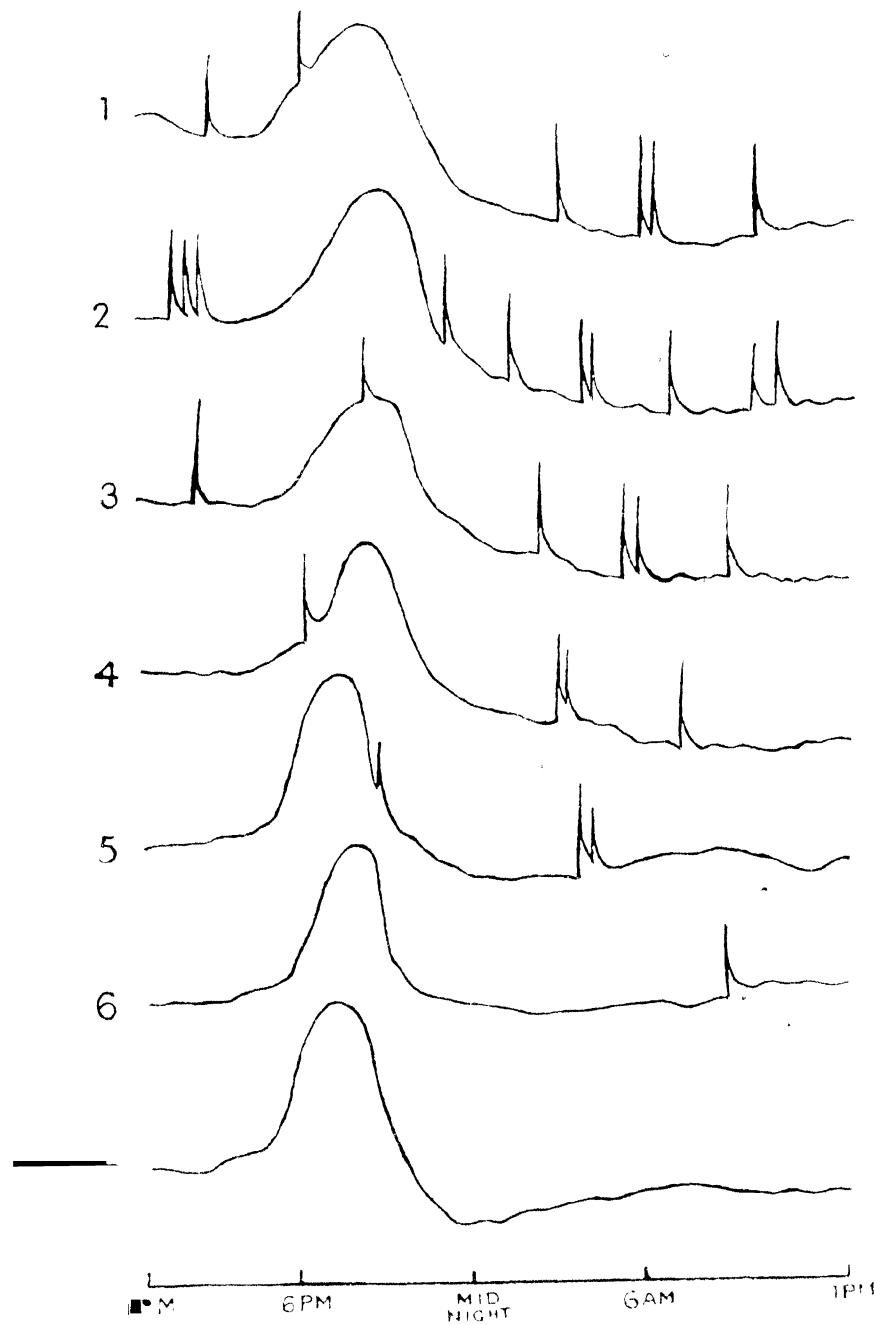
does not bring about similar reaction. It may be that the young top leaf, due to its close proximity to the apical bud, receives greater supply of hormones to be utilized there in its rapid developmental process.

Though autogenic responses appear to occur randomly, diurnal frequency curve, computed from data of a large number of records, indicated two maxima, one between 2 and 4 p.m. and the other between 9 and 11 a.m. It is rather difficult to interpret how these particular periods are more favourable for the expression of the autogenic activity. The curve also indicated comparatively lower activity during the night than at daytime. Though the occurrence of the responses cannot be correlated with any one of the environmental factors, light and temperature may have some relation with the enhanced activity during the day. If the autogenic response is brought about as a result of some reaction produced by hormone, the intensity of that reaction is likely to vary due to the influence of such environmental factors. In this connection reference may be made to the work of Yin (1941). In working out the periodic concentration of auxin in the leaf of *C. papaya* he obtained two maxima in the periodic curve, one in the afternoon and the other in the morning. His interpretation is that the maximal concentration in the afternoon is due to an active production of auxin in light, and the maximal concentration in the morning as being due to transformation of auxin precursor to auxin. In the pulvinus of Mimosa the possibility of such periodic variation in the concentration of its hormone content cannot be excluded.

B.5. *Electric potential associated with excitation process in* M. pudica.— In his monograph ' The Nervous Mechanism of Plants ', Bose has studied the different types of response, mechanical, electrical, released in *M. pudica* on stimulation. Because of the simplicity in the method of observation of mechanical responses, this method has been mainly utilized by Bose to study the intermediate processes which modify the intensity of response to a given stimulation. The velocity of propagation in *M. pudica* is generally studied by this method. Bose has at the same time made some fine studies on the electric responses in plant tissues by using the comparatively quick recording Einthoven string galvanometer.

In the present investigation, a multiple pen electric recorder has been employed. Electrodes from two points on the plant tissue, e.g. petiole, are connected to two of the pen recorders ; the other terminals of these recorders are earthed. Both the pens record on the same strip of recording paper, enabling a time sequence study of the responses. The objects of study are (i) the character of each record whether monophasic or diphasic, (ii) time difference between the corresponding records made by the two pens, from which information can be obtained (a) on the direction in which the excitation current is travelling and (b) on the velocity of this current of excitation.

B.5.1. *Experimental arrangement.*—Two electrodes are placed at A and

B on the petiole of a *M. pudica* pulvinus leaflet unit. The stimulus is applied to one of the subpetiole leaflets. The electric excitation potential as it traverses along the petiole from the leaflet to the pulvinus and in the reverse direction is measured at two places A and B on the petiole.

[*Note.*—To understand the nature of the records obtained, it is necessary to describe briefly the nature of the mechanical response observed when one of the leaflets is stimulated by local heating or by a cut. If a thermal stimulus is applied to the tip of a subpetiole leaflet, in the outside one, in a bundle of four leaflets connected to the petiole by subpulvini, the following sequence of events is observed :

(i) The pinnule of the stimulated leaflets starts closing from the tip downwards, (ii) after some time the main pulvinus drops, (iii) again after an interval the pinnule of the three remaining leaflets starts closing from base towards the tip; usually the leaflet next to the one originally stimulated closes earlier than the others. Here we have an illustration of what Bose rightly calls a slow speed reflex arc, comprising the following stages: (*a*) an afferent impulse, starting from the tip of the stimulated leaflet, traverses basipetally through the petiole till (*b*) it reaches the main pulvinus which drops, releasing an efferent impulse, (*c*) this outgoing impulse traverses the petiole now in the reverse direction (*d*) on reaching the subpetioles, the latter respond by closure of the pinnules acropetally from the bottom to the tip.]

The present investigation was planned mainly to follow the electric action potential accompanying the ingoing and outgoing impulses of the reflex arc in *M. pudica*.

The results obtained are given below:

B.5.2.1. *Moderate stimulation.*—A glowing tip is made to touch lightly the end tip of a pinnule. The resulting record of the propagation of the electric action potential as recorded at points B and A on the petiole is given in Fig. 8. A large monophasic action potential which judging from the time records passes from B to A. Superposed on it is a small biphasic current.

(*a*) The velocity of propagation of the electric action potential arising out of a moderate stimulus is equal to the

$$\frac{\text{distance between } B \text{ and } A}{\text{time interval}} = \frac{7 \text{ mm.}}{0 \cdot 5 \text{ sec.}} = 35 \text{ mm./sec.}$$

(*b*) Superposed on this monophasic response is a biphasic one, at both the electrodes B and A. They are to the right of the monophasic ones and more advanced to the right of B than of A.

We interpret these as fast moving outgoing electric response currents from the stimulated pulvinus. The dotted line P represents the instant of fall of the main pulvinus.
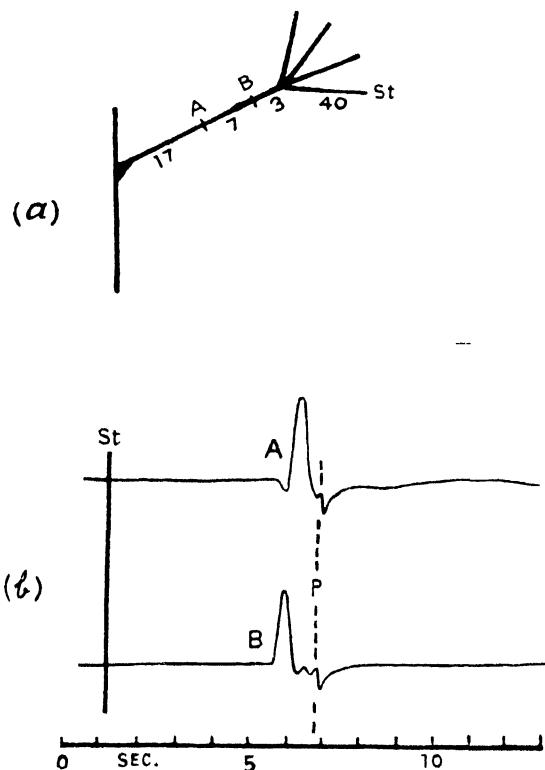
FIG. 8.   Transmission of action potential along the petiole of Mimosa.

(a) Showing contact points on the petioles A and B, led to the electronic amplifiers and pen recorders; (moderate) stimulus applied at st, on the subpetiole; the numerical figures indicate distance in mm. of the intervening spaces.

(b) Time difference between two responses at B and A ¼ sec. indicating velocity of transmission 35 mm. per sec.   Action potentials indicated at A and B are 60 and 50 mV respectively.   Note simultaneous diphasic response at A and B, with contraction of pulvinus indicated by dotted line.

It is further to be noticed that there is no record of any monophasic outgoing action current from the pulvinus.   This may be due to the conducting tissue of the petiole being put in a refractory state after the passage of the ingoing monophasic current.   If it is so, then it is not understood through which portion of the petiole tissues the biphasic current is propagated.   We have further to assume that it is this biphasic action current flowing in the reverse direction along the petiole which causes the closure of the three remaining pulvinules.   It is not possible however with the present records to determine the velocity of its propagation; it can only be said that its rate of propagation is faster than that of the monophasic action current.

B.5.2.2. *Strong stimulation resulting from a deep cut on a subpetiole.*—Fig. 9. ·Monophasic response; from the position of the response curve along the time base, we conclude that the monophasic current is an efferent one proceeding outwards from the stimulated pulvinus. The monophasic current is preceded by a biphasic one, emanating from the pulvinus. From our present view on the mechanism of propagation of excitation potential in living tissues the passage of such an excitation current is followed by a refractory period. If this is accepted to hold good for plant conducting tissues, we cannot explain how a biphasic and a monophasic action potentials can almost simultaneously travel along the same conducting channel of the petiole.

Another point which is not understood now is why there is no record of a
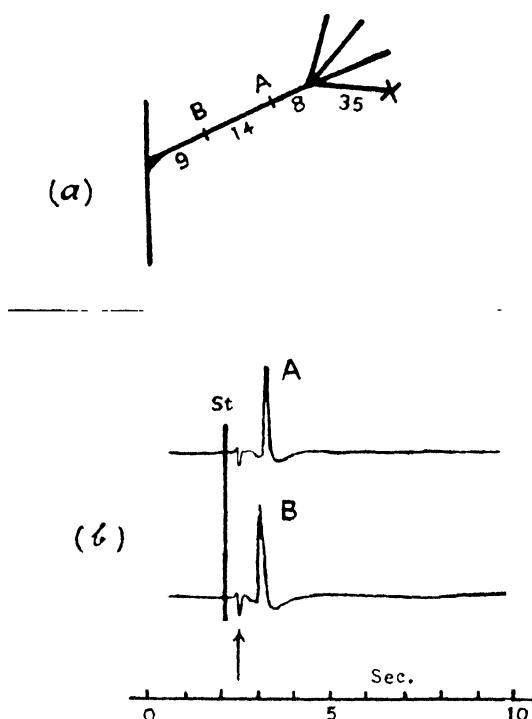
FIG. 9. Showing efferent impulse reflected from the pulvinus.

    (*a*) *B* and *A*, basal and apical connections respectively, in the petiole led to two different amplifiers and pen systems; stem cut stimulus applied at the cross mark on a subpetiole; numerical figures indicate distance in mm. of the intervening spaces.

    (*b*) Stimulus applied at *st*; simultaneous appearance of diphasic response due to fall of pulvinus is indicated by arrow mark; note appearance of action potential first at the basal connection *B*, and then at the apical connection *A*.

monophasic action potential accompanying the passage of the afferent excitation from the cut leaf tip along the petiole to the pulvinus.

These are some of the as yet unexplained observations in the present investigation. Using higher amplifying and faster recording unit which is being assembled, it is expected new results will become available from which a more consistent interpretation of the electric correlate of the excitation phenomena in the pulvinar petiole unit of *M. pudica* will be possible.

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PLAGUE IN INDIA

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ABSTRACT

Plague which for centuries ravaged many parts of the world, as also India, is now receding from this country. It prevailed in India in a severe form during the first quarter of this century and then waned gradually to almost vanishing point by the end of the present decade. Nearly the whole of the present-day knowledge on the subject has been acquired during this period, the workers in India contributing to its major share.

Historically the disease is an antiquated one affecting primarily the rodent kingdom and the man getting it from them. In the Indian history the disease is mentioned in *Bhagvata Purana* (1500–600 B.C. ?). It appeared at least thrice in pandemic form, India being severely involved in the last one and Europe in the second with 'Black Deaths' for well-nigh three centuries.

The paper describes the various aspects of infection, carriers, antigenic structure of the organism, prophylaxis, control measures, etc.

INTRODUCTION

Plague which, as one of the most dreaded diseases, haunted the world with its epidemic and pandemic ravages for centuries killing millions of human beings is now on the way to recede from the Indian soil as it did in Europe towards the end of the seventeenth century. In its recent history plague prevailed in India in a serious epidemic form during the first quarter of this century, but it gradually tailed off nearly to a vanishing point towards the end of the present decade (1950–60). Almost the entire knowledge about plague as it stands today has been acquired within this period and it is one of the diseases in which the workers in India have probably contributed a major share. The purpose of this communication is to briefly describe the present status of this knowledge and the relationship it bears to the ultimate conquest of this great pestilence as far as its present position in India is concerned.

HISTORICAL SUMMARY

From the evolutionary point of view all diseases at some stage or the other were prevalent among the animal kingdom from which man got them by legacy or contact. Plague is only one of them, which man even now gets from animals, particularly the rodents. Only under special circumstances is the infection transmitted from man to man when the organism aberrates into a specially virulent form causing pneumonic plague and serious havoc

among human beings. But in so doing it undergoes the risk of complete destruction with the cessation of the epidemic. It can only reappear from rodents or other animal reservoirs.

The first human epidemic on record according to Wu Lien-teh was the outbreak among the Philistines in 1320 B.C. It was characterized by the appearance of emerods in their secret parts as described in I Samuel, V and VI, in the Bible. Some workers, however, doubted this interpretation, but it is not so material when it is believed that the plague was in the animal kingdom and still is, and whenever opportunities were propitious, it propagated among the human beings like so many other diseases. Indian scriptures like the *Bhagvata Purana* (1500–600 B.C. ?) gives this disease an earlier antiquity by referring to the deaths caused by an epidemic disease preceded by an epizootic among rats. Men were warned to quit their houses when a rat fell from the roof, jumped about as if it was drunk and died. Similarly it is not essential to know whether the origin of plague was in Central Asia or Central Africa, as described by some of the workers. The moot point is wherever mankind gave up the wild living and started to settle as organized families and tribes the disease started affecting them in groups at times following contact with infected animals either in their wild state or during their attempts at domestication.

More reliable accounts of plague are, however, found beginning from 200 B.C. and it appears from the writings of Rufus, Physician at Ephesus about A.D. 100, that the plague was prevalent in Libya, Egypt and Syria during and before his time, probably as far back as 300 B.C. (Wu *et al.* 1936). The more recent happenings are, however, important in the understanding of the secular trend, the extent and intensities of the problem in the immediate past and the chronological order of events which have led to the present situation. Of the three classical pandemics, the first one occurred during the reign of the Emperor Justinian (A.D. 542). It started from Pelusium, a great trading centre in lower Egypt, from where it spread through North Africa to the Roman Empire on the one side and to Syria, Palestine and Constantinople on the other, and thence to other parts of Europe and Asia reaching London in A.D. 662. It lasted for 50–60 years and killed about 100 million people.

The second pandemic started in the fourteenth century (A.D. 1347) from Caffa in Crimea and spread to China and India on the one side and Asia Minor and North Africa on the other. It was imported to Geneva through the army and from there it spread to other parts of Europe reaching England by A.D. 1349. The disease then ravaged Europe for well-nigh three centuries under the horror of what was known as ' Black Death ' taking a toll of 25 million human lives. Evidently pneumonic and septicaemic manifestation with bloody sputum, characteristic cyanosis and skin haemorrhages leading

to black or blue spots on arms, thighs and other parts was quite frequent although the bubonic form must have been more preponderant as in rural England (Greenwood 1911). Retrogression started in the seventeenth century from West to East completely leaving the European stronghold by 1841.

This prolonged pandemic in Europe ushered in new changes in the idea to causation of diseases, their spread and treatment, by revolting against the old system and bringing in the new, what is known as Renaissance in historical term. Quarantine laws were first passed in A.D. 1374 by Count Bernardo of Reggio and by the Venetians in 1403, followed by land cordons and sanitary improvements in the towns.

Millions died in Asia as well, during this pandemic, and India, too, experienced several epidemics. Starting from the north-western part of the country it spread to the central and southern parts and declined towards the close of the seventeenth century leaving behind endemic foci in the foot-hills of the Himalayas in the district of Garhwal and Kumaon where the disease was locally known as 'Mahamari' and persisted for a long time.

The third pandemic (A.D. 1894) was traced to the reappearance of plague in South China at Yun-nanfu in 1866 from where it reached Canton and Hong Kong by 1894. It then spread far and wide through the marine transport and involved almost all countries except the main lands of Europe by the year 1900. It reached Calcutta in 1895 and Bombay in 1896 and spread from there to almost all parts of India except Orissa, Assam and Eastern Bengal. It prevailed almost unabated till 1918, with a total of 10·25 million deaths. The peak year was in 1907 with 1,315,892 deaths (sp. death rate 5·16 per 1,000). The great epidemic of pneumonic plague in Manchuria started in 1910 and by 1919 all plague epidemics began to decline everywhere except in Java and East Indies. A noteworthy feature of this period of decline was the persistence of plague in some endemic foci, particularly in Asia and India and in some cases in wild rodents as in South Africa, California, Iranian Kurdistan, South America, etc. The spread of infection was rather rapid in this pandemic compared to the slow extension in the second, due to the improved transport and communication facilities.

CHRONOLOGICAL HISTORY OF EPIDEMICS OF PLAGUE IN INDIA

1500–600 B.C.   Record in *Bhagvata Purana*.

A.D. 1031-32   Plague reached India from Central Asia following invasion of Sultan Mahmoud (Arabian chronicles).

A.D. 1325   Plague in Malabar following invasion of Mahmoud Toghulak and again after Timur.

A.D. 1403   Sultan Ahmed's army was destroyed by plague epidemic in Malwa.

A.D. 1617   Plague reported during the Moghul Emperor Jehangir's reign from the Punjab, Ahmedabad, Surat and Deccan and some other parts of India—described by Edward Ferry, Ambassador to the Moghul Court.

A.D. 1707   Plague in Berhampur.

A.D. 1812–21   In Kathiawar, Gujerat and Cutch—supposed to have been imported from Persia.

A.D. 1836–38   In Merwar and Rajputana—which is known as *Pali* plague.

19th century   Endemic foci in the north near Rawalpindi, in Kumaon and Garhwal (U.P.).

A.D. 1895   In Calcutta—diagnosed bacteriologically on the 17th April, 1898, by Dr. Neild Cook, imported from Hong Kong.

1896   In Bombay, first diagnosed on the 13th October, 1897. From here plague spread rapidly to most parts of India.

1907   Peak year of plague in India with 1,315,892 deaths.

1926-27   Severe epidemic in Hyderabad and Deccan.

1947-48   A temporary rise of incidence in several old foci in India.

Epidemic Behaviour of Plague

The historical review shows that plague allowed to pursue its natural course assumed pandemic form periodically and spread in different directions originally from the endemic home of Central Asia or Central Africa and involved distant parts of the globe. It then receded to its original home leaving behind islands of endemic centres in the various parts of the world, particularly in submontaneous areas. Depending upon the means of communication and conditions favourable for its reception, it took a century or more to reach distant parts of the globe. A number of centuries passed before the disease showed signs of regression, as it also took more or less a century for the process to be completed. With faster and more frequent means of communication this behaviour also changed and the spread of infection in the other pandemic became accelerated manifolds. The important point is that the areas last involved were usually the first to recover unless they presented favourable grounds for sylvatic plague. During pandemic prevalence the disease exhibited epidemicity of varying degrees in different areas according to local conditions. Occasionally in the inter-epidemic periods plague was introduced into non-endemic areas but failed to establish itself and fizzled out in a year or two as in Assam recently (Seal and Bose 1957). Thus plague exhibited two types of secular periodicity, viz. (i) long-term periodicity with intervals of centuries and continuing for centuries or part of a century when once started at such intervals, e.g. recurrence of pandemic plague in the sixth, fourteenth and twentieth centuries, and (ii) short-term

periodicity—a tendency to renew at short but irregular intervals. This is apart from concurrent seasonal periodicity.

The phenomenon cannot be explained in terms of fortuitous happenings or ephemeral local conditions. It rather comes as an outcome of the working of the biological laws. It would be wrong to regard squalor, poverty and absence of sanitary safeguards as the determining cause of pandemic because neither are they confined only to plague epochs nor are they peculiar to plague-infected countries. It is insufficient to ascribe it to spread of infection along a trade route that has probably been operative since time immemorial, while it cannot be denied that the direction and rapidity of spread may be affected by the prevailing methods of transport and communication. The belief expressed by some workers that the ousting of the black-rat by the brown was the cause of decline of the second pandemic may perhaps be partially substantiated although the history of plague in Bombay, Calcutta and other places in India shows that these two species can live together in amity and play their part with fine impartiality in determining the occurrence of plague epidemic of great intensity. What we have found is that whenever the proportions of black-rat as also of X. cheopis have been reduced or lessened due to better housing and sanitary conditions, the incidence of plague has also come down or greatly reduced. A comparative study of ward 8 (plague affected) and ward 10 (plague unaffected) in Calcutta by the author during 1954–60 also supported this view.

Again, that the emergence of pandemic is due to a gradual rise of the infection quantum to the epidemic flash point has so far remained as a speculation based on probabilities rather than on experimental proof. Similarly it is a surmise that the decline of pandemic and its gradual retreat to its indigenous home or old (primary) foci is dependent upon the rise of communal immunity as a result of which the disease disappears first from those areas where the conditions are least favourable to its persistence and from other parts of the world outside its indigenous home. According to this assumption the essential cause of the long-term periodicity or of pandemic plague is ascribed to the rise and fall of herd immunity of rodents rather than to the recurrent happenings and ephemeral local circumstances. It may be mentioned here that in 1948-49 plague not only reappeared in Calcutta alone but also in many other towns and cities where plague was absent or at least quiescent for a long time, e.g. Bombay, Lucknow, Gaya, Dhanbad, Asansol, etc. Simultaneously a tendency to recrudesce in places like Hyderabad, Mysore, etc., had been noted. Similar phenomenon was noticed during the last influenza pandemic. The point is how these events or phenomena can be explained or interconnected.

In the author's experience a probable explanation is that when an infection reaches almost to a stage of extinction the organism undergoes a mutation

in virulence, or toxicity or both as a biological process for the preservation of species. By this procedure the effect of rise of immunity or resistance against the existing strain in the community is avoided, and the freshly-emerging organism may then have another lease of life through the epidemic or pandemic which it causes. This is what had happened in influenza in 1957. The phenomenon can be compared with that of locusts. But the question is how the inter-epidemic period is actually bridged over in plague. This was, however, the subject of study by the author in the period 1954–57 which will be referred to later.

ENDEMIC AREAS

World

India, Burma, Java, Indo-China, China, Madagascar, South, Central and East Africa, Equador, Brazil, Bolivia, Peru, Venezuela, Argentina, Iraq, Iran, Western Arabia, Western United States (mainly wild rodent foci), Hawaii (see Map I).

India

Sub-Himalayan foci in the Punjab, Uttar Pradesh and North Bihar, Madhya Pradesh, Hyderabad (now in Bombay), Bombay, Mysore and Madras (Sharif 1951 ; see Map II).

The important landmarks in the development of knowledge about plague is given in Appendix 1.

FACTORS INVOLVED IN THE EPIDEMIOLOGY OF PLAGUE

The factors involved in the epidemiology of plague are mainly five, namely the organism, the rat, the flea, the man and the environment, the man being excluded in epizootology. The interaction of these various factors determines the appearance, rise, fall, disappearance and periodicity of epidemic, in other words, its epidemicity and endemicity. Also plague being primarily a disease of the rodents human epidemic is generally always secondary to rat epizootic except the isolated instance of pneumonic plague in Manchuria.

RECORDS OF PLAGUE IN INDIA SINCE 1895

India got involved very early in the third pandemic in 1895-96 and the peak was reached in about 9-10 years in 1907. Since then the mortality from plague has been one of continuous fall (Graph I) as will be seen from the decennial mortality figures given in Table 1 and Graph II.

Table I shows that starting from a specific mortality rate of 181·3 per 100,000 during the first decade of plague epidemic in India it came down to 1·8 per 100,000 in the last decade (1949–58). The average annual deaths

MAP II

Plague endemic centres of the Indo-Pakistan subcontinent (Sharif 1951).

TABLE I

Mortality from plague in India during the period 1898–1957 arranged in decennial periods

Period	Total death from plague	Total population in each period *	Specific mortality rate per 100,000	Plague death as percentage of total deaths, 1898–1957	Average annual percentage of total deaths
1898–1908†	6,032,693	3,291,915,990	183·3	47·47	4·32
1909–1918	4,221,529	3,155,926,382	133·8	33·22	3·32
1919–1928	1,762,718	3,283,195,808	51·9	13·40	1·34
1929–1938	422,880	3,619,458,716	11·7	3·33	0·33
1939–1948	268,596	3,965,924,896	6·8	2·11	0·21
1949–1958	59,059	3,287,649,065	1·8	0·46	0·05
Total	12,767,475	-	—	—	—

* Population was first calculated for each year by intercensus correction and then added together for different periods. Since 1948, population of the areas forming Pakistan was excluded.

† The first period taken was of 11 years.
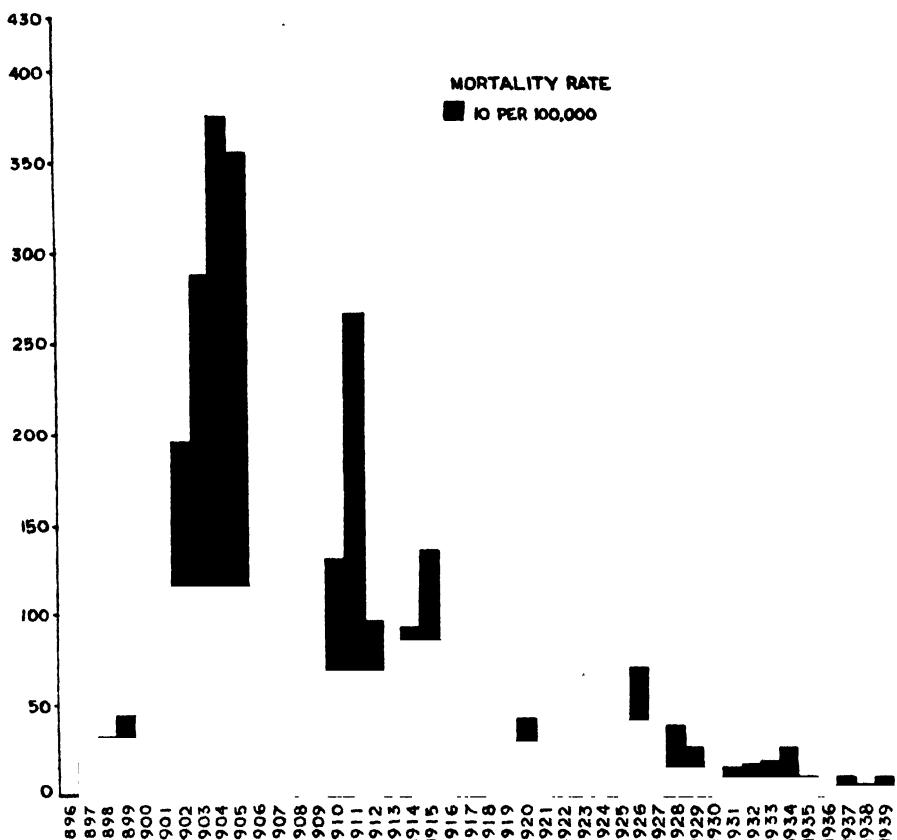
GRAPH I

Plague mortality in India per 100,000 population (1896–1939).

due to this disease also came down from 4·32 to 0·05 per cent of total deaths registered. No death from plague has been reported since 1958 except for few stray cases from Mysore and Madras. It must, however, be mentioned that although there was a continuous decline of incidence since 1939 it fluctuated quite alarmingly between 1945 and 1947 and as high as 78,937 human deaths due to plague occurred in India in 1947 as compared to 10,577 deaths in 1942 (*see* Table II and Graphs III and IV). The chronology of State-wise incidences of plague between 1939 and 1957 representing the years 1939, 1942, 1947, 1950, 1953 and 1957 have been diagrammatically shown in six maps included in Diagram I. Plague is thus receding from India. Whether it is a prologue to the final disappearance as it did in Europe, or it is only a phase in the secular trend or it is due to certain measures taken, is a point for consideration. It is on the correct assessment of the present situation that the nature of the steps to be taken now and in future will depend. It
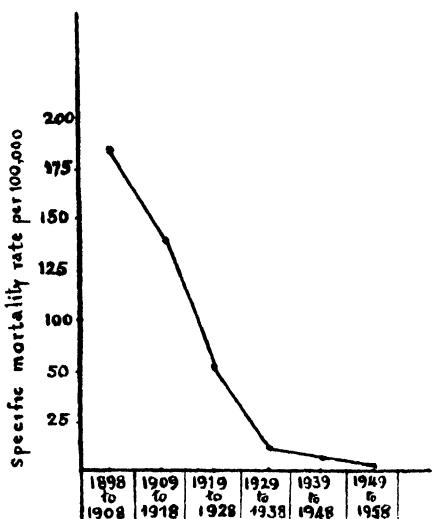
GRAPH II

Decennial death rates of plague per 100,000
population in India (1898-1958).
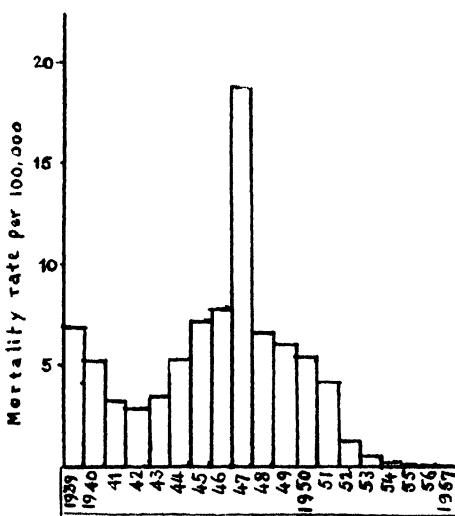
GRAPH III

Plague mortality per 100,000 population in India (1939 57).
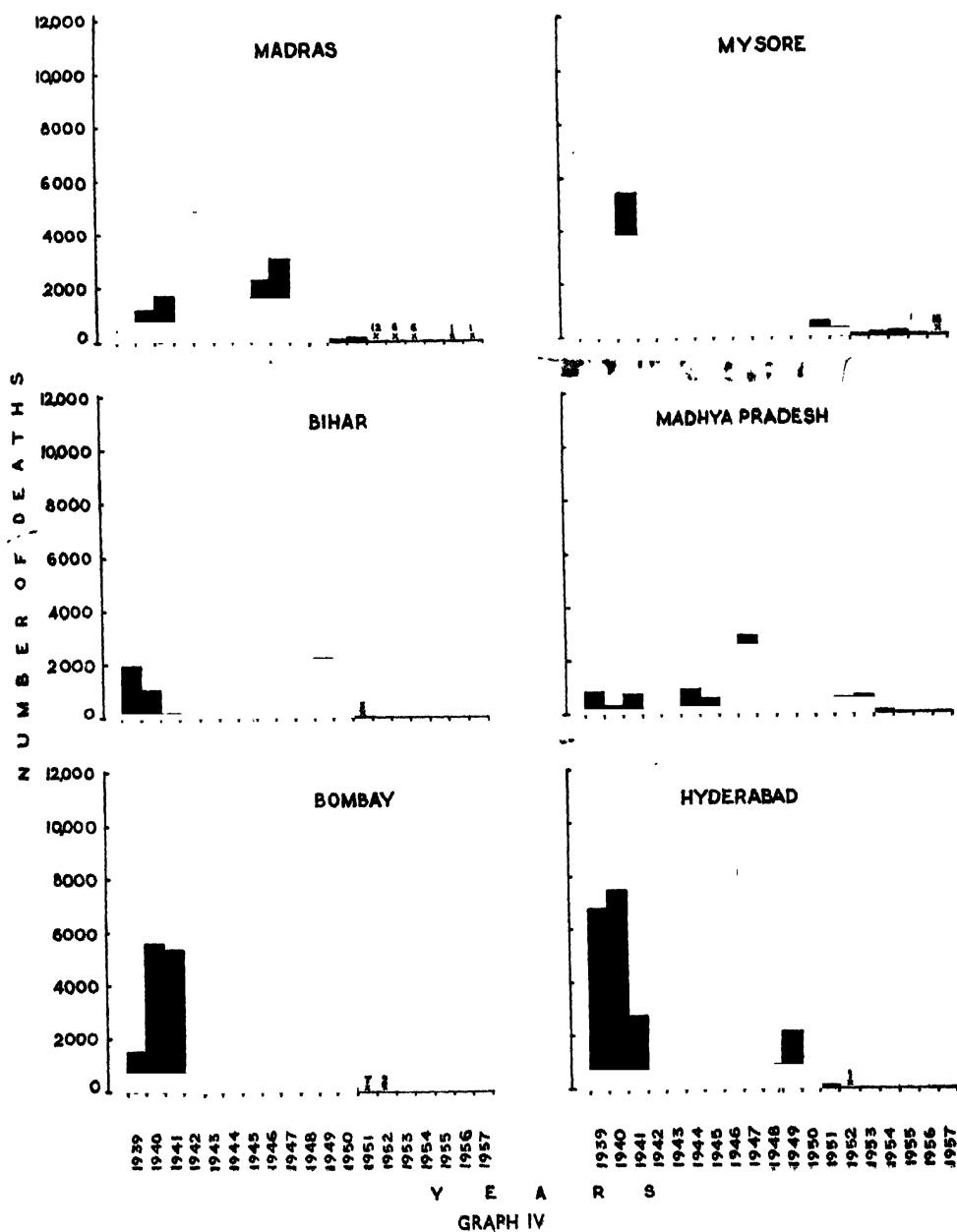
GRAPH IV

Plague deaths in different States in India (1939–57),
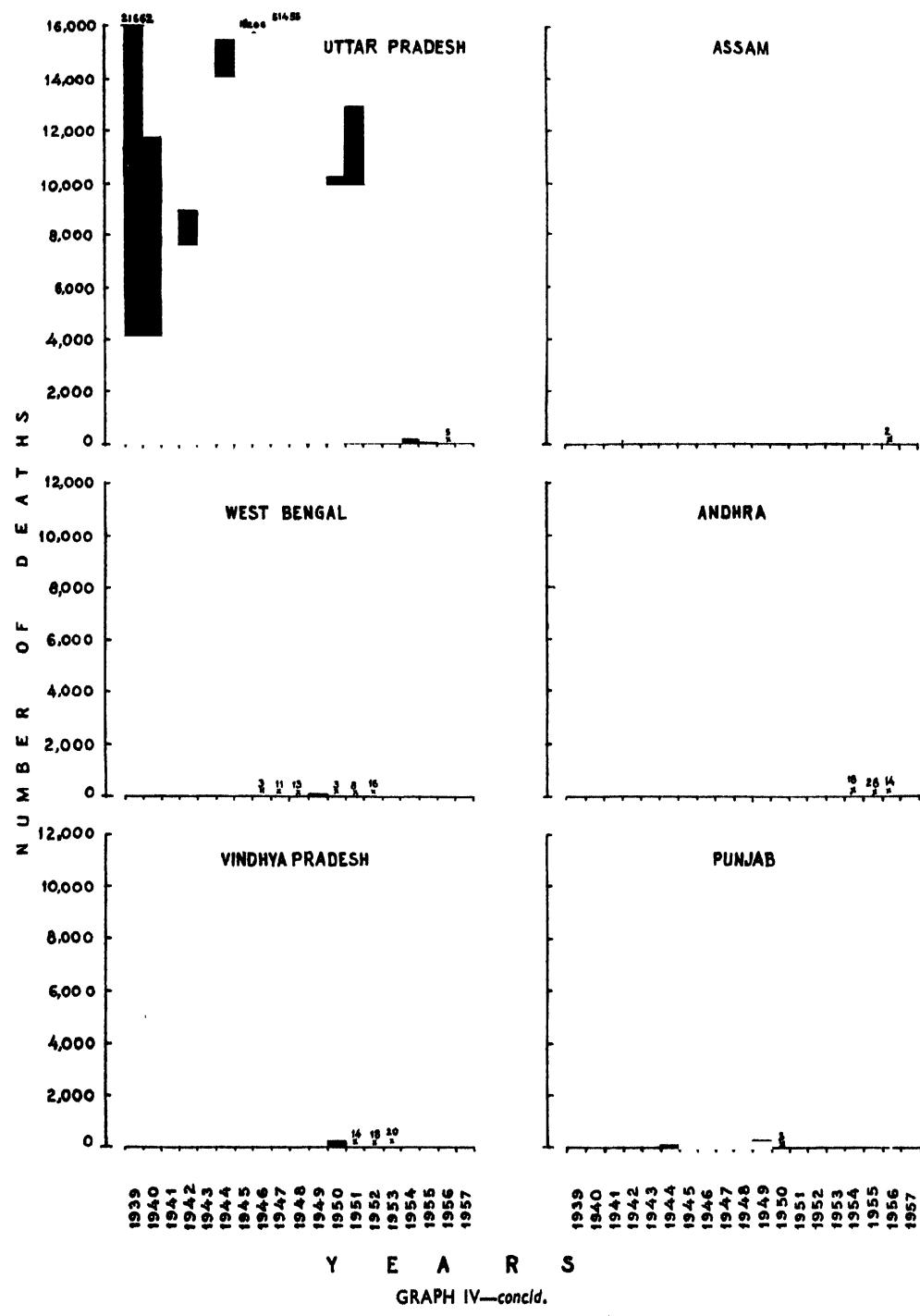
GRAPH IV—*concld.*
Plague deaths in different States in India (1939–57).

TABLE II

Annual deaths from plague in different States in India during 1939-57

Years	Andhra	Assam	West Bengal	Bihar	Bombay	Hyderabad	Madhya Pradesh	Madras	Mysore	Uttar Pradesh	Vindhya Pradesh	Punjab	Others	Total	Rate per 100,000
1939	—	—	—	1,938	1,472	6,758	852	324	2,352	21,662	—	—	9	26,257	6·88
1940	—	—	—	1,040	5,573	7,500	283	1,169	2,593	11,725	—	—	9	19,799	5·13
1941	—	—	—	129	5,311	2,713	761	1,725	5,417	4,035	—	—	22	11,984	3·08
1942	—	—	—	108	680	657	129	701	3,776	8,953	—	—	6	10,577	2·67
1943	—	—	—	266	715	1,498	144	4,885	3,886	7,556	—	—	11	13,578	3·38
1944	—	—	—	834	2,514	5,263	910	1,738	5,357	15,454	—	61	14	21,526	5·29
1945	—	—	—	1,523	11,779	6,631	575	1,644	8,016	14,024	—	203	3	29,751	7·21
1946	—	—	3	8,689	3,405	4,026	189	2,254	2,894	18,206	—	245	6	32,997	7·84
1947	—	—	11	13,204	3,081	1,791	2,902	3,078	1,502	51,455	—	1,905	8	78,937	18·61
1948	—	—	10	2,142	1,305	811	2,560	978	1,128	13,722	—	211	16	23,191	7·02
1949	—	—	57	2,155	1,139	2,103	3,479	151	982	9,875	—	241	19	20,197	5·76
1950	—	—	3	1,449	146	719	5,568	42	255	10,231	196	3	201	18,813	5·33
1951	—	—	8	3	7	98	513	60	542	12,959	14	—	12	14,178	3·97
1952	—	—	16	0	2	1	575	12	272	3,107	18	—	20	3,905	1·08
1953	—	—	0	0	0	0	679	6	56	762	20	—	—	1,385	0·378
1954	18	—	0	0	0	0	54	6	115	157	—	—	—	296	0·08
1955	28	—	0	0	0	0	0	0	137	29	—	—	—	194	0·052
1956	14	2	0	0	0	0	0	1	52	5	—	—	—	74	0·019
1957	0	0	0	0	0	0	0	1	15	1	—	—	—	17	0·0044

Rajasthan, Kashmir and Jammu—no plague since 1950.
Orissa and other States—no plague.
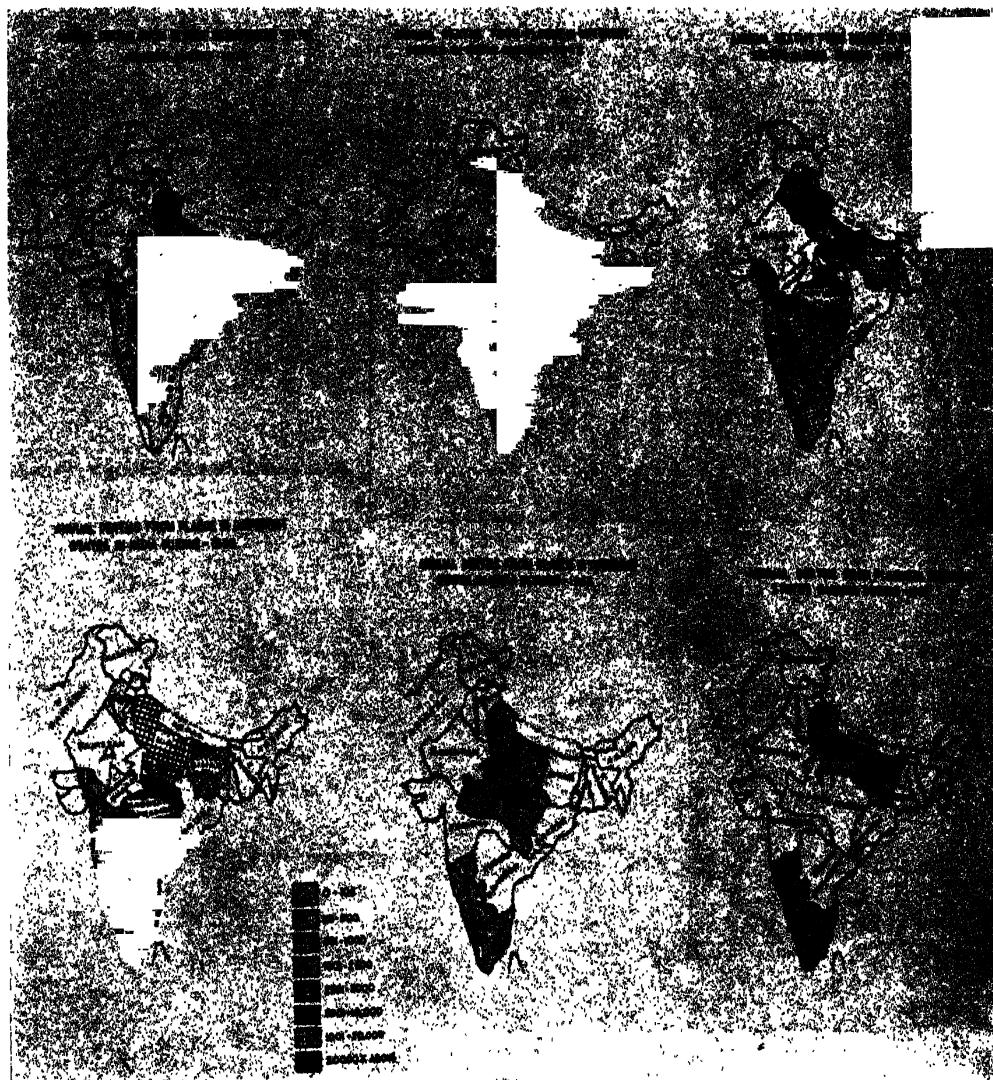
DIAGRAM I

Annual deaths from plague in different States in India during 1939, 1942, 1947, 1950, 1953 and 1957.

has already been noted that only recently in 1956 a new area in the State of Assam was involved but fortunately aborted.

The portioning of plague deaths on State-wise basis between July 1898 and June 1932 is given in Table III.

It will be seen from Table III that the main brunt of the onslaught fell upon three States mainly, the Punjab, Bombay and U.P., followed by· Bihar

TABLE III

The Province-wise distribution of plague mortality between July 1898 and June 1932

Province	Mean population census, 1901, 1921, 1931	Total plague deaths, July 1898– June 1932	Per cent of all India total	Mortality per 1,000 of mean population
Punjab	21,142,793	3,489,123	28·7	165·0
Bombay	19,877,756	2,460,132	20·2	123·8
U.P.	47,164,594	2,911,837	23·9	61·7
Bihar and Orissa	34,692,676	1,113,937	9·2	32·1
C.P.	13,991,863	468,165	3·8	33·5
Hyderabad..	12,855,934	425,302	3·5	33·0
Mysore	5,970,446	314,673	2·6	52·7
Rajputana ..	10,330,957	282,312	2·3	27·8
Madras	42,168,483	227,184	1·9	5·4
C.I. Agency	7,653,893	149,941	1·2	19·6
Burma	5,970,446	149,427	1·2	11·8
Other areas	38,477,465	109,597	0·9	2·8
Bengal	46,109,157	68,809	0·6	1·5

and Orissa, C.P., Hyderabad and Mysore (of British period) in serial order. The mortality per 1,000 mean population was highest in the first three States mentioned above. Much appears to depend on the social conditions of the patients and attention and nursing available. For instance, in the Hong Kong epidemic case the fatality among the overcrowded, indifferently-fed and unclean Chinese amounted to 93·6 per cent, it was 77 per cent among the Indians, 60 per cent among the Japanese and only 18·3 per cent among the Europeans. Similarly in the first Calcutta epidemic, according to Crake (1908), the mortality varied between 91·2 and 94·5 per cent among the Hindus and between 94·2 and 96·2 among the Muslims whereas the same amongst the Christians was between 51·3 and 53·3 per cent only. The chance of recovery was better in men than in women. The disease also assumed less severity in the vaccinated than in the unvaccinated.

CLINICAL FORMS OF PLAGUE IN INDIA

Plague is essentially bubonic in India. True septicaemic plague is rare except in case of accidental laboratory infection. Primary pneumonic plague is also rare. Generally, it happens after lung-involvement in a bubonic-septicaemic case leading to plague pneumonia and subsequent contacts of such cases develop primary pneumonic plague. Such outbreaks were reported in India (Seal 1949a; Seal and Prasad 1949) but they generally remained confined to one or few families only. In fact, the incidence of pneumonic plague remained below 1 per cent and never exceeded 3 per cent of plague deaths in any year since 1895.

Pneumonic form

Wu Lien-teh (1926) tried to explain how this pneumonic form arose. Recently Meyer and Larson (1959) carried out some interesting experiments on the cross-infection among primates (*M. rhesus* and *M. cynomolgus philippinensis*) intra-tracheally infected with plague infection and succeeded in establishing pneumonic infection in 11 per cent and cervical bubonic-septicaemic infection in 58 per cent of those exposed by contact. Henderson (1959) also made similar experiments with guineapigs. Although small particles of infection caused broncho-pneumonia cross-infection was rare and, if at all, ended in septicaemia and death but not broncho-pneumonia. Sokhey suggested that pneumonic plague may be a double infection probably with influenza virus. The author, however, thinks that there is no necessity for such a postulation as once the pulmonary plague develops the organism becomes transmissible through droplets like any other lung infection and thus starts pneumonic plague, but other lung conditions may predispose to pulmonary involvement in a bubonic-septicaemic case.

URBAN AND RURAL PLAGUE IN INDIA

Plague is both urban and rural in India, the predominance being of the latter. It appears that plague has failed to gain a foothold in many of the towns of India, perhaps due to untoward climatic conditions and lack of efficient vector (as in Madras and Assam). Regular heavy annual flood may be responsible for keeping certain States like East Bengal free from plague. Another factor which may play an important part is the types and proportion of rodent distribution. With suitable flea vector, larger proportion of susceptible *R. rattus* will facilitate easier and quicker spread among human beings than with other rodents and vice versa. Development of acquired immunity among commensal rat following prolonged outbreaks may either prevent an outbreak or allow simmering zootic plague. Again the urban plague may persist for several years once it is entrenched there in the presence of large rodent population and suitable vectors. If such towns or cities are of commercial importance having traffic connection with other towns and rural areas they often spread plague infection and create secondary plague distributing centres for further propagation of plague. In one essential aspect the urban plague may differ from rural plague in that the latter may be dependent upon commensal, peridomestic or even wild rodent infection and is usually initiated by importation of infection.

The problem of persistence of plague in rural areas still remained a problem for study in India. Kunhardt (1912) tried to explain it by formulating the hypothesis of 'incomplete' and 'complete' plague. In the former case the outbreaks come to an end in the first year before the entire rodent

population has been covered and it reappears in the next season and so on till the coverage is complete, resulting in elimination of the susceptibles and development of immunity among the survivors. Actually there were villages in the Cumbum valley (Madras) where plague used to appear year after year. However, Baltazard *et al.* (1958) in their recent observation in U.P. concluded that plague was not endemic there, but there was continuous shifting of the infection from one place to another through contiguity or colony infection among wild rodents, eventually infecting the commensal rats of a village on their path and causing epizootics and human cases. While this observation needs to be confirmed the author believes that multiple factors are involved and the same factor may not be operative in all places equally and the environment, climate and season may also play their respective roles. Besides, all epizootics are not followed by human cases.

Plague may also spread from the affected rural areas to towns through grain traffic or communications as observed by Pollitzer in China (Pollitzer 1954). Again plague may shift from one village to another in the succeeding year and come back to the first village at two or three years' interval (area-wide endemicity ?). According to Sharif (1951) the slow type of epidemic, killing fewer rats, persists longer than the severe epidemic causing heavy rat mortality.

SEASONAL INCIDENCES

In bubonic plague the optimum conditions of temperature and humidity as observed under Indian conditions are roughly represented by a mean temperature of 68°–77° F. in association with a relative humidity of the order of 60–70 per cent (some local differences according to different geographical situations may be noticed). There is marked decrease in the incidence of plague with mean temperature rising above 90° F. The largest number of cases occur in the year of highest relative humidity. The seasonal incidence of pneumonic type in Calcutta between 1904 and 1907 is shown in Graph V. The influence of the season is on the numerical prevalence and longevity of rat fleas and on the multiplication of plague organism during the intra-corporeal phase either in rats or in fleas. Rats rarely develop septicaemia below 10° C. (50° F.).

It is thus held that the climate factors, by reason of their influence upon the transmission of infection, are capable of determining the season of the year in which the epidemic of this disease would most likely occur. For instance, at higher latitude the atmospheric temperature attains the critical level only during the late summer and early autumn (as in Kashmir) or at precisely the season of the year when plague epidemic is liable to occur at that latitude. A decrease of latitude is generally associated with earlier occurrence (as in Madras). In the sub-tropical region, on the other hand, where either the temperature or the humidity factor is unfavourable during

GRAPH V
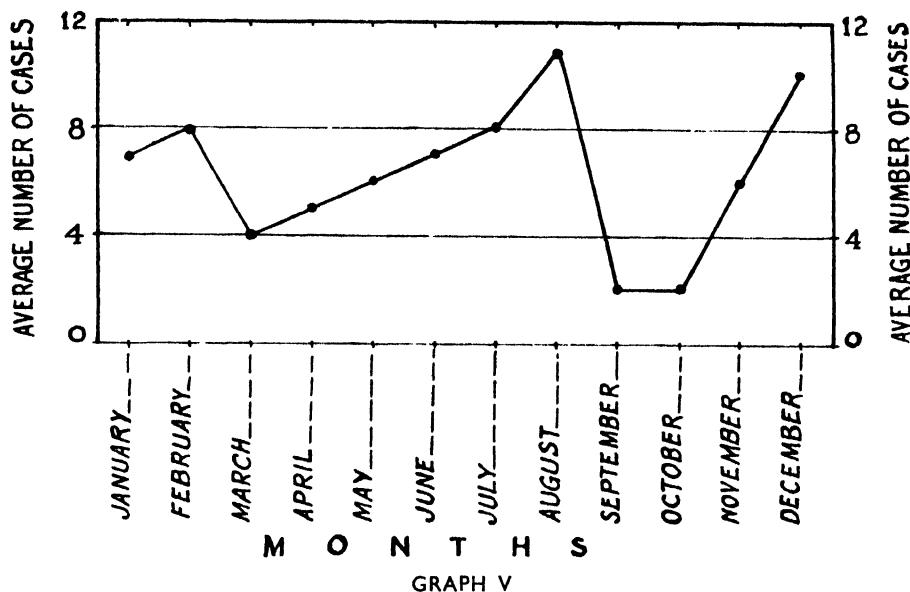
Pneumonic plague in Calcutta (1904–7).

the summer, the plague epidemics have a vernal periodicity (as in northern India).

An analysis of the first 20 years' records of plague epidemics by the author (Seal 1949b) in different parts of India since 1896 (Graph VI) suggests two principal seasons of epidemic intensity in a year, viz.—(1) A broad-based single wave, starting in autumn or late autumn and rising to a peak in March or April. In this category is included generally the northern Indian provinces with the Punjab in the north-west and Bengal in the north-east. The city of Bombay (but not the State) has also similar characteristic. (2) Double waves. In this category four characters could be discerned, viz. (a) main autumnal wave with a secondary rise in the early months of the year—as in Hyderabad and Mysore States; (b) main spring or late spring wave with secondary rise in the autumn, e.g. Kashmir and N.W.F.P.; (c) almost equal waves in the autumn and spring, e.g. Bombay province and states, C.P. and Central India and (d) the main peak occurring in the early months of the year with occasional secondary rise in the autumn, e.g. Madras province. In brief, the peak of the spring wave is delayed more and more as we move from the Bombay area towards north, north-east or to any higher altitude like Kashmir and also there is a tendency to replace the double waves with a single one. On the other hand, the peak comes earlier as we move towards the south or south-east. For instance, in some outbreaks in N.W.F.P. the peaks occurred in May and even in June whereas it was in

SEASONAL DISTRIBUTION OF PLAGUE MORTALITY IN DIFFERENT CITIES & PROVINCES IN INDIA

(Monthly means and medians expressed as percentage of yearly totals)

GRAPH VI
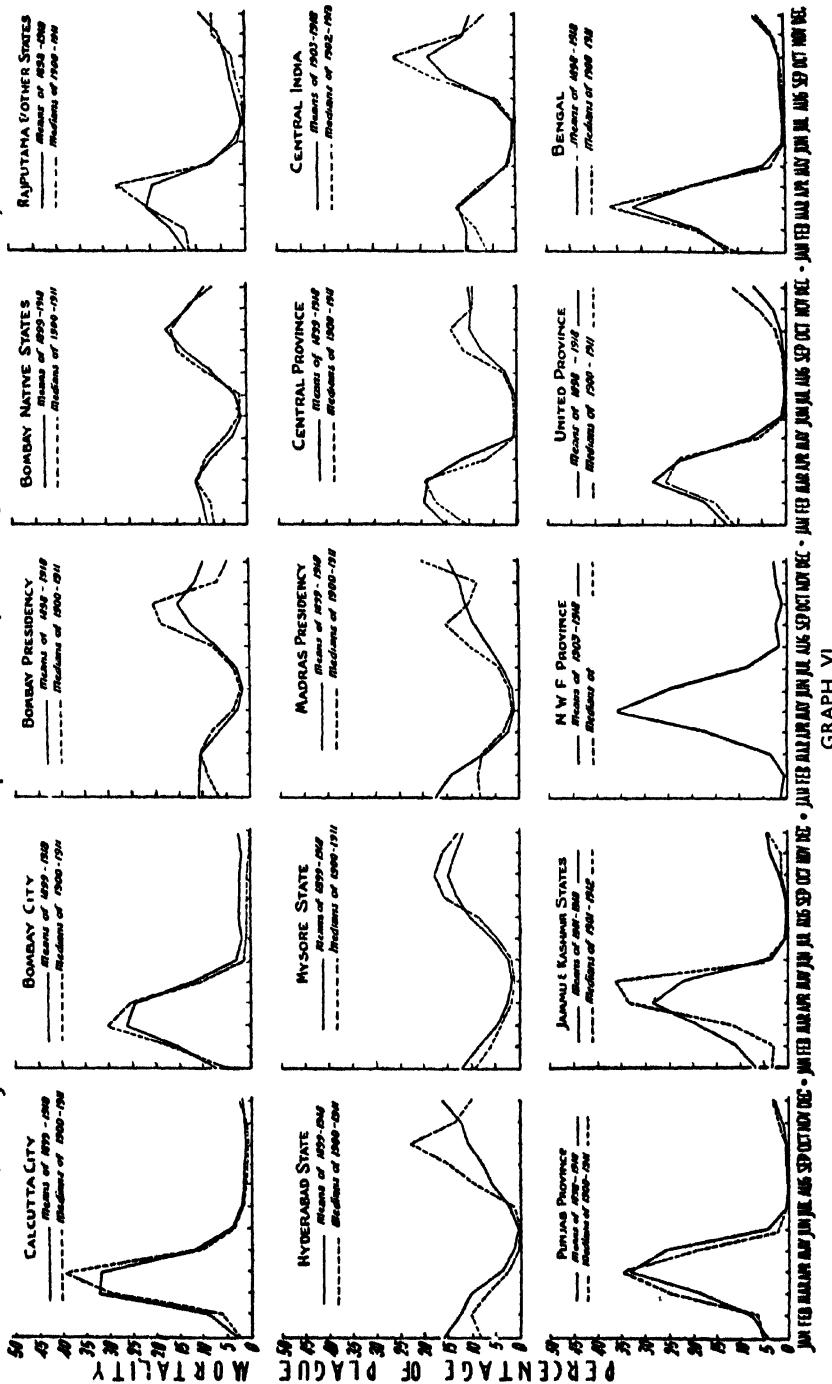
January or February in Madras province. The two months which show the least incidence in India are June and July, and also January and February where the winter is severe (*see* Table IV). In Calcutta out of 20 epidemic waves since 1896, 4 reached the peak in March, 12 in April and 4 in May. On the whole, in the northern Indian belt the maximum plague mortality occurred between March and May and the disease tended to die out with the onset of hot weather, i.e. high temperature with reduction of humidity, both of which are inimical to the longevity of rat flea and to its power to transmit infection. The most favourable period is therefore autumn and spring, particularly the latter. This period has been found to be related to the normal growth curve of the *X. cheopis*, the vector fleas (Seal 1955, 1958a).

RESERVOIRS OF INFECTION

The rodents which have been found associated with plague infection are generally classed as (1) wild rodents, (2) commensal or domestic rodents and (3) peridomestic or semi-domestic rodents, according to their ecological behaviour. Only domestic rodents are involved in pandemic plague and in most of the epidemics. The role of wild rodents seems to be mainly in the maintenance of plague on long-term basis with occasional transfer of infection through semi-domestic rodents to the domestic rodents and thus to perpetuate epizootics among the commensal rodents and eventually among human beings.

Wild rodents

Since 1929 the list of rodents other than that of rats and mice, known to suffer from natural plague and also of those suspected of carrying the infection, has grown consistently and lately the W.H.O. has published a comprehensive list of rodents and lagomorpha containing more than 200 species or subspecies involved in the natural plague infection. During this period foci of wild rodent plague have been defined in Central Asia, South-East Russia, South Africa, East Africa, South America, U.S.A., and recently Iranian Kurdistan. The characteristics of the principally-involved species such as movement range, susceptibility and resistance, role of interaction, effect of density, seasonal incidence of plague and trend of epizootics, modes of spread of infection and the interrelationship between wild rodent and rat plague have been studied.

As far as India is concerned, the earlier workers could not secure any convincing evidence for the independent existence of wild rodent plague in India except occasional positive findings in *Funumbulus pennati* (Simond 1898; Hirst 1922), in *Tatera indica* and *Mus booduga* in the Cumbum valley of Madras by George and Webster (1934) and in *Tatera indica* and *Mellardia meltada* in Bombay by Sharif and Narasimham (1941). On the other hand,

TABLE IV

Number of plague outbreaks in any month in the period 1898–1918 in different parts of India

Province or city	Years of study	J	F	M	A	M	J	J	A	S	O	N	D	Months of lowest incidence	Start of rise	Remarks
Bombay City	20	—	—	7	13	—	—	—	—	—	—	—	—	June-July or Nov.-Dec.	Nov.-Dec. or Jan.	During first six years rise was in February; in remaining years generally in January
Bombay Province	21	3	4	11	2	—	—	—	—	2	16	1	—	June-July	July-Aug. and Jan.	Double rise: autumn and spring
Bombay States	20	1	3	11	4	—	—	—	—	3	13	2	—	June-July	July-Aug. and Jan.	Double rise: autumn and spring
Hyderabad States	21	4	6	2	—	—	—	—	—	—	9	3	4	June-July	July-Aug. or Jan.	Irregular double rise: fall of peak often continued until June-July or next year
Mysore States	20	7	2	—	—	—	—	—	3	4	7	4	—	May-June	July and Jan.	Generally continuous fall from autumn peak
Madras Province	20	12	4	—	—	—	—	—	1	4	1	2	1	May-July	Aug.-Sept. or Dec.-Jan.	Double rise in 8 instances with main rise in winter
Central Province	20	1	8	5	—	—	—	—	—	1	7	3	2	June-July	Aug. or Jan.	Double rise in 10 instances; equal number of peaks in autumn and winter
Central India	16	1	5	9	7	—	—	—	—	2	2	2	—	May-July	Aug. or Jan.	Double rise in all years
Rajputana States	21	—	1	10	7	—	—	—	—	1	11	1	—	June-July	Aug.	Double rise in 4 instances only. Main rise in spring
Punjab	21	—	—	3	15	3	—	—	—	—	1	1	1	July-Aug.	Sept.-Oct.	Peak only in spring
Kashmir	18	—	—	2	7	7	—	—	—	—	1	1	—	July-Aug.	Oct. or Nov.	Double rise in three instances only, but peaks very small. Main rise in the late spring
North-West Frontier Province	10	—	—	—	1	5	2	—	—	—	1	—	2	Jan.-March	March or Oct.-Nov.	Double rise once only with very small peak in December
United Provinces	19	—	—	18	1	—	—	—	—	—	—	—	—	June-July	Sept.	Peak only in early spring
Bengal (including Bihar)	20	—	—	13	6	1	—	—	—	—	—	—	—	June-July or Aug.-Dec.	Aug.-Sept. or Jan.-Feb. (later years)	Peak only in spring
Calcutta	20	—	—	4	12	4	—	—	—	—	—	—	—	July-Oct. or Aug.-Dec.	Nov.-Dec. or (later years)	Peak only in spring

Rao (1947) in Hyderabad found *Tatera indica* very susceptible and *R. rattus* resistant. Previously Sokhey and Chitre (1937) came to the same conclusion. Recently, however, Baltazard *et al.* (1958) found infection in *Tatera indica* in certain parts of Uttar Pradesh.

Peridomestic and commensal rats

Infection was, however, more commonly found among the peridomestic rats like *B. bengalensis* (Hossack 1906), *B. indica* (Indian Plague Research Commission 1907 and 1910) or *B. bengalensis* and *B. malabarica* (George and Timothy 1941). The author also during his investigational work on the recent Calcutta outbreak (1948–51) found evidence of dormant infection among the peridomestic rats, *B. bengalensis*. Chowdhury (1957) isolated such infection from the rat. The three important species among the commensal rodents (murinae) which are usually involved in plague epizootis are *R. rattus*, *R. norvegicus* and *Mus musculus* and occasionally *Suncus murinae* (musk rat). Although a fairly intensive study has been made about their ecology, there is enough scope for further study depending upon the area, geographical, climatic, soil and physiographical conditions. Among the important items of ecological studies made may be mentioned: (1) breeding habits, (2) population dynamics, (3) movements, migration and

TABLE V

Types of rats and their percentage distribution

City	Year	Observer	R. rattus	R. norvegicus	B. bengalensis	Bandicota	Others
Calcutta	1906	Hossack	14·0	26·0	60	—	—·
	1936	Rao	13·5	22·0	27·3	—	37·2
	1948–50	Seal and Bhatta-charji	13·3	9·0	75·8	1·7	
	1956	Chowdhury	7·3	9·0	79·9	0·3	
Bombay	1910	Indian Plague Research Commission	66·2	28·7	1·0	—·	4·1
	1929–30	Webster and Chitre	79·1	11·0	9·9	—	—
	1956	Deoras & Gokhale (Haffkine Institute, Bombay)	22·9	15·9	49·2	—·	12·0
Madras	1910	Indian Plague Research Commission	49·4	—	—··	—	50·6
	1931	King and Pandit	98·8	—·	—	··	1·2

transportation, (4) relative distribution of different species in a locality, (5) resistance and susceptibility, and (6) maintenance of infection. In regard to the breeding habits the author's observation in Calcutta shows that there is an accumulation of rat population during the first three months of the year, the peak month being April or almost coinciding with the plague epidemic season (Seal 1958a). The study of rat movements showed that their movement is mainly intramural and intercolonial but they trace their path back to the colony when released seven or eight miles away. The author also believes that there is metastatic movement also at the initial stage of an epizootis which helps propagation of infection to even distant parts (Seal 1958b).

In recent times a changing pattern of distribution of rodents has been noted in Calcutta, Bombay and Madras as will be seen from Table V.

MOVEMENTS OF RATS IN RELATION TO DISSEMINATION OF PLAGUE INFECTION

Under normal conditions the commensal rats in the urban area move within a very narrow orbit (Davis 1951; Bhattacharji and Seal 1954; Kartman and Lonergan 1955). In the rural areas they generally have a strictly limited home range but, as noted by Venables and Leslie (1942) and Davis (1951), they are able to make seasonal migration and, according to Macchiavello (1948), they are apt to undertake progressive migrations covering wide distances. Some workers also have noted mass migration, though not common. In this connection the author made an interesting observation in the city of Calcutta during the last 1948–50 outbreak of plague (Seal and Bhattacharji 1960a and b). Suspecting the dispersal of rats at this very early stage of the epizootis carrying infection simultaneously to distant parts experiments conducted with 'marked' rodents caught from the field and released at long distances from the places of their habitat showed that the rats so removed had a tendency to go back to their home colonies, even though they had to cover long distances for the purpose. He also observed that though there was considerable intermingling of neighbouring colonies, ordinarily the distance covered did not exceed 50 yards and was never more than 200 yards. In agreement with the observation it was found that the larger percentage (61·4 to 72·0 per cent) of confirmed human plague cases could be explained on the basis of rat deaths detected between 50 and 200 yards and 22 per cent beyond 200 yards; only 10·4 per cent remained unexplained. Therefore wherever plague-affected rats were detected it was necessary to apply intensive control measures within an area of 200 yards.

The problem of passive transference of rats through marine and land traffic, particularly of food grains, cotton, jute, etc., is well known.

RESISTANCE OF THE RAT POPULATION

As a result of a series of outbreaks of plague in a locality the surviving rat population acquired herd resistance against the disease due to active immunization and possibly also to natural selection and a stage is reached when the major section of the population acquires resistance, and the plague completely ceases there for the time being. This was one of the ways how plague subsided in Bombay. The results of resistance test of Bombay rats carried out at the Haffkine Institute are given in Table VI.

TABLE VI

Species	Percentage of mortality					
	Bombay					Calcutta
	1952	1953	1954	1955	1956	1953-54
R. rattus	12·6	13·7	12·1	16·0	7·5	87·0
R. norvegicus	- -	5·8	3·3	2·6	2·2	94·7
B. bengalensis Kok	77·6	82·5	76·7	70·0	75·2	97·6

It will be seen that between 1952 and 1956 there is a progressive increase of resistance in *R. rattus* and *R. norvegicus* but these two species are being replaced by the more susceptible species, *B. bengalensis* Kok, which now constitutes nearly 50 per cent of the rat population in the city. In Calcutta only *R. rattus* is found partially resistant but the population has recently been reduced to half of what was in 1906 or 1948. Recently Haffkine Institute was asked to regularly examine the rodents collected from different states in India for resistance and except those sent from the Madras city others have been found fairly resistant. This observation is in favour of the view that development of herd resistance of rats in different areas in the country has been partly responsible for the disappearance of plague, at least temporarily.

VECTOR FLEAS

The two subfamilies of fleas called *Pulicidas* and *Coratephyllidae* are of great importance so far as plague is concerned. As with the rodents, the various species of fleas parasitizing wild, peridomestic and commensal rodents in different parts of the world, harbouring or suspected of harbouring plague infection, have been listed. Also the bionomics and ecology of this common species have been studied. A large volume of information is already

available regarding their growth and development, host selectivity, breeding habits, hibernation, biting habits, relative distribution in different parts of the country (local distribution), infectivity, mechanism of transmission of plague infection (blocked fleas, faeces, per os, mechanical, etc.), climatic and environmental influences, vector incidence and vector efficiency, longevity, role of infected and uninfected fleas, transportation of fleas in the spread of plague, role of wild rodent fleas, interchange of fleas between wild and domestic rodents, and so on. Besides, the role of other insects including the *Pulex irritans*—the human flea—has also been studied.

In India, the species of fleas found are : *X. cheopis, X. brasiliensis* and *X. astia* and *Ctenocephalus felis* and *canis*. The first two species are efficient vectors of plague infection while *X. astia* is a poor vector. In fact, one of the reasons of absence of plague in the Madras city given by King and Pandit (1931) was the absence of *X. cheopis*, the only species found being *X. astia*. The types of fleas and their distribution in the five cities of India at different times are given in Table VII.

TABLE VII

Types of fleas and their distribution in different cities of India

Places	Observer	Per cent of distribution				
		X. cheopis	*X. astia*	*X. brasiliensis*	*Cteno-cephalus*	*P. irritans*
Calcutta	Strickland and Roy (1930) ..	40·0	60·0	—	—	—
	Rao (1936) ..	40·4	59·6	—	—	—
	Seal and Bhatta-charji (1948–50)	34·4	65·6	—	4 fleas	—
Bombay	Cragg (1920) ..	49·5	49·8	0·7	—	—
	Cragg (1922-23)	53·1	45·8	1·0	—	—
	Webster and Chitre (1930) ..	69·6	27·9	3·3	—	—
	Deoras and Tonpi (1956) ..	76·3	23·7	—	—	—
Madras	King and Pandit (1931) ..	5·6	94·3	1 flea	1 flea	1 flea

Table VII shows that the distribution of *X. cheopis* and *X. astia* varies in different places, Calcutta having predominance of *X. astia* (65·6 per cent), Bombay of *X. cheopis* (76·3 per cent) and Madras almost wholly *X. astia* (94·3 per cent). A changing phase is also noticeable particularly in Bombay. In 1920, the city had almost equal distribution between the two fleas, a change was noticed in 1930 and it is still being maintained, *X. cheopis* constituting

at least three-fourths of the flea population. Besides, *X. brasiliensis* which was prevalent in low percentage has now practically disappeared. It may be mentioned here that the Madras city has always been free from plague. Along with the change in rat population in the city, the resistant *R. rattus* is being gradually replaced by the more susceptible *B. bengalensis*, and with this high rate of *X. cheopis* population the city of Bombay is running a potential risk of plague recrudescence.

Recent studies on the bionomics of fleas (Seal and Bhattacharji 1960*a*) show that while the reproduction goes on all the year round *X. cheopis*, the vector species, has two principal waves of growth—one in the winter months immediately preceding or covering the epidemic wave of plague, also indicated by higher flea index, and the other a smaller one during the rainy season, whereas *X. astia* has only one main wave of growth in the rainy season, extending to autumn. Deoras and Tonpi (1956) had similar experience in Bombay. While the peak of *X. cheopis* incidence was between March and May that of the *astia* was between September and December, the corresponding incidence of the other species being lowest during those periods. A concomitant change took place in the sex ratio also. For instance, during the peak incidence of *X. cheopis* the females increased greatly in population than the males and the proportion of *X. astia* was just the reverse at that time. In regard to the biting habits, *X. cheopis* is normally a poor feeder on man but the propensity definitely increases during the winter and spring both for human and rat blood. Furthermore, a comparative field study of the plague-free and plague-endemic wards in a city shows that the relative distribution of the species of rodents and fleas definitely influences the epizootic conditions (Seal 1954*b*).

Resistance in flea population

During recent years a number of communications reporting on the resistance of certain insects to DDT have been reviewed by Busvine (1957). Kilpatrick and Fay (1952) reported resistance in certain fleas to DDT but not to 5 per cent chlordane, while Wilson *et al.* (1957) noted that the fleas had become resistant to chlordane also. In India, no actual resistance of flea population was reported till 1958. But a few human cases having occurred in certain villages in Mysore, the local flea population was examined at the Malaria Institute of India and was found to have developed fair amount of resistance. In this regard a systematic study was undertaken by Patel *et al.* (1960) in the Bombay State. They observed high degree of DDT resistance in the Poona strain of *X. cheopis* being 19 to 5,000 times more resistant than the Satara strain. The interesting fact is that these strains became partially resistant to BHC (8 to 16 times) and dieldrin (2 to 3 times) although these

insecticides were not used in Poona. This resistance has apparently developed due to the spraying of wettable DDT (0·5–1 gm. per m²) 2 to 3 times in the malaria season of the year. The concentration of DDT having deteriorated soon led to the development of resistance among the survivors. Nevertheless, it appears that the fleas in other areas may have been similarly affected and it is now essential to test them for resistance, so as to adopt suitable steps before the situation deteriorates further.

Mechanism of persistence of infection

Four years' continuous natural transmission experiment through *X. cheopis* carried out by the author (Seal 1957, 1960c) with both susceptible and partially immunized *R. rattus* and *B. bengalensis* on the lines of experimental epidemiology after Greenwood *et al.* (1936) has led to the conclusion that following an epizootis the plague infection may be maintained for prolonged periods in an inapparent or sub-clinical form in the commensal or peridomestic rodents and that depending upon the environmental and other ancillary conditions these inapparent foci (usually in the spleen) may lead to a sort of relapse with bacteriaemia followed by clinical plague and death or recovery with rise of immunity. Fleas play only the role of a vector in the matter of transmission and a temporary reservoir at best. It was also noted that in the perpetuation of infection, organisms of low virulence played more important role than the virulent organisms. Thus this experiment has provided some evidence to show that one of the mechanisms by which the inter-epidemic period is bridged over is the carrying over of infection, by the partially-resistant commensal and peridomestic rodents in the urban areas.

THE PLAGUE BACILLUS

Since the discovery of plague bacillus by Kitasato (1894) and Yersin (1894) the problem related to its cultural methods, morphology, virulence, toxicity, antigenic structure, biochemical behaviours, serology and its differentiation with similar organisms particularly *P. pseudotuberculosis* have been studied and many obscure points clarified. Some studies have also been made of its character during epidemic and inter-epidemic periods. But the essential difference between the organisms of bubonic and pneumonic plague has not yet been elucidated.

(i) *Morphology and growth characteristics*

The so-called normal and involution forms as described by the earlier workers have now been found to be caused purely by unsuitable extrinsic conditions, as in older infection, primary buboes, decomposed carcasses or

unsuitable culture medium. Cultivated in a properly nutritive medium such as enriched casein hydrolysate broth or agar (Seal and Mukherji 1950; Seal 1950), sheep or rabbit blood agar (Sokhey 1939) or beef heart agar (Meyer 1948), etc., the growth is uniform, smooth and profuse. There is hardly any lag phase or auto-agglutination. On the above solid media the freshly-isolated virulent organisms show smooth, convex, viscous and often dew-drop-like colonies with or without a fringe. Only a rough and avirulent form shows a little different colony and morphological character. Another important point that emerged out of the above studies is that the plague organism undergoes quick dissociation on repeated subculture particularly in an un-suitable medium and gradually loses its protective antigenic properties. The classical stalactite growth is therefore an indication of roughness and degeneration of the organism.

(ii) *Nutrition of plague bacillus*

The nutrition of plague bacillus is the essential prerequisite for all plague studies because on this depend its cultural, morphological, biochemical, antigenic, virulence and other differential characters. It does not grow in ordinary agar or broth medium unless seriously degenerated. Certain additional nutritive and accessory growth factors are necessary for its full development including its antigenicity (Rae 1939, 1940). This was the basis of the author's own work in some of his investigations (Seal and Mukherji 1950; Seal 1950, 1951a, d, e), because it is extremely important from the point of view of maintenance of the organism without losing its virulence and anti-genicity for the preparation of vaccine. Jackson and Burrows (1956) have recently showed the virulence enhancing effect of iron on non-pigment (relatively avirulent) mutants of virulent plague strains. The enriched casein hydrolysate medium of the author (Seal and Mukherji 1950) not only contains iron but also traces of Ca, P, Mg and small amount of liver extract. One of the criteria of nutritive medium is the virtual absence of the so-called lag phase as noted in case of the enriched casein hydrolysate broth.

For maintenance of the organism freeze-drying in 5 per cent gum acacia has given excellent results in the author's hand (Seal and Habbu 1943). Alternatively, point culture on 5 per cent rabbit blood agar, slant or stab-culture in solid medium of the same composition kept sealed in cold room, maintains the full character of the organism for more than a year.

(iii) *Antigenic structure*

Prior to 1940 the plague workers were facing certain difficulties in regard to the physical, chemical, serological and immunological properties of plague

bacillus and the related organism like *P. pseudotuberculosis*. The particular difficulty was in regard to its serological behaviour and its differentiation with *P. pseudotuberculosis*, as the success of field endemiology and the detection of reservoirs during the inter-epidemic period largely depended on them. Investigation made by various workers from time to time (Sokhey and Maurice 1936; Schutze 1939; Bhatnagar 1940; Jawetz and Meyer 1944; Seal 1950, 1951*b, c, d, e*, 1952 and 1953; Devignat 1951; Girard 1953; Burrows and Bacon 1956) have greatly clarified the position.

The plague bacillus is now primarily divided into (*a*) virulent and (*b*) avirulent forms. The latter is again subdivided into (i) protective and (ii) non-protective strains, based on animal tests. The bacillus loses its virulence not only by cultivating it in artificial culture medium but also in the immune rats during the inter-epidemic period as observed by the author (Lal and Seal 1949; Seal 1958*c*). Serologically, the author (Seal 1951) was able to differentiate the virulent from the avirulent non-protective plague and pseudotuberculosis organisms but no differentiation could be made between the virulent and avirulent protective strains except by animal test and quartz ultraviolet spectrographic readings of the respective specific proteins. The other methods of differentiation between the plague and pseudotuberculosis organisms are: culturally, *P. pseudotuberculosis*, unlike virulent *P. pestis*, grows easily on agar slope, shows motility in stab culture, ferments glycerol and rhamnose, reduces malachite green and methylene blue, and so on (Seal 1952). Englesberg *et al.* (1954) concluded that virulence was determined by the quantitative relationship between envelope and toxin production.

Recently, however, Burrows and Bacon (1956) by a special technique have discovered two additional antigens called V and W which can differentiate virulent from the non-virulent plague strains being present in the former and absent in the latter. In a further extension of the study Burrows (1959) obtained two additional determinants of virulence, namely P (pigment positive) and Pu (purine producing). The V antigen is related to the property of the related organism by which it resists phagocytosis by mouse polymorphs. He has also studied both freshly-isolated and laboratory strains of *P. pseudotuberculosis* for the same antigens and found that the main difference between the two organisms was the absence of Fl in the pseudotuberculosis strains. The other three antigens are the same as *P. pestis* except that in some strains any one of them may be absent and, like *P. pestis*, the virulence can be enhanced with iron under similar conditions. Accordingly the serological antigenic structures of the plague and pseudotuberculosis organisms stand as follows :

Organism	Antigens
P. pestis virulent	Fl +*, VW +, P +, Pu +, common rough somatic antigen
P. pestis avirulent protective	Fl +, VW —, P + or P— } Pu +, common rough somatic antigen
P. pestis avirulent non-protective	Fl —, VW —, P + P— or P + } Pu —, common rough somatic antigen
P. pseudotuberculosis virulent (fresh)	Fl —, VW +, P +, Pu +, ⎫ flagellar smooth and somatic smooth (type and group specified)
P. pseudotuberculosis virulent (laboratory)	Fl — VW —, P +, Pu +, ⎭ common rough smooth

* Fl of Bakor *et al.* is the same as A of Seal and relates to the envelope substance.

Devignat (1951, 1958), on the other hand, classified all the world strains into three noso-geographical varieties, based on their biochemical behaviour and on clinico-pathological effects (virulence). These are :

(i) *Var orientalis*, which does not ferment glycerine, transforms nitrates into nitrites and produces nitric acid in broth without nitrates and causes late septicaemia in mice; found in India, South China, Morocco, Madagascar, Indonesia, South Africa and U.S.A. This type corresponds to Berlin and Borzenkov's (1938) oceanic strains.

(ii) *Var mediavelis*, which ferments glycerine, does not transform the nitrates and does not produce nitric acid; seems to be to some extent pneumotropic; found in South-East Russia and Kurdistan.

(iii) *Var antiqua*, which ferments glycerine, transforms the nitrates but does not produce nitric acid; shows a tendency to provoke septicaemia; found in North-East Asia, North China and Belgian Congo.

Capsule and envelope

Controversy still exists in regard to the term envelope antigen and the capsule. Kurauchi and Homma (1938) called it 'capsular antigen', and Chertnik (1940) 'membrane antigen'. Previously Rowland (1914) postulated that capsule was present in certain circumstances only but Sokhey (1940)

concluded that the plague bacillus possessed a capsule under all circumstances and the envelope seemed to be only an unstained capsule. In 1951, Amies called the envelope as nothing but partially well-developed bacterial capsule.

The author (Seal 1959a, c) carried out some intensive study on this problem and finally succeeded in defining both of them in the same specimen of virulent *P. pestis* by an ingenuous method of staining procedure (*see* photograph, Pl. XVIII). Secondly, while the protective avirulent plague strains like the Java and Madagascar (EV) strains also contain the envelope substance the non-protective avirulent strains are without it.

Toxin of the plague bacillus and enzyme studies

Rowland (1910) originally suggested that the plague bacillus had an endotoxin associated with the soluble protein. A few workers also suggested that it is a mixture of metabolic and disintegration products of the bacillus. Clinically also, the plague patients show various degrees of toxaemia. According to Girard (1941) and Girard and Sander (1947) plague endotoxin is similar to exotoxin, being thermolabile and convertible into toxoid, while Baker *et al.* (1952) claimed that Fraction II of specific soluble protein of plague bacillus (isolated between 0·4 and 0·57 saturation of ammonium sulphate) contained the toxic fraction but the identity of this substance was not clear. A very useful work has recently been done by Ajl *et al.* (1955, 1958). These workers either used casein hydrolysate mineral glucose medium (CHMG) and the Tjs strain or the autolysates of agar grown, acetone-killed dried bacilli of virulent strain 1951 P or attenuated EV 76. Partial purification was achieved by fractionation with 0·35 to 0·70 saturation of ammonium sulfate in the final purification by continuous flow paper electrophoresis or by electrochromatographic methods. The intravenous LD 50 in mice varied from 0·1 to 0·3 μg. The toxin could be denatured by physical or chemical agent with decrease or eventual disappearance of toxicity, but the formalin-treated toxin retained its ability to react with its specific antiserum while the enzyme-hydrolysis with trypsin, chymetrypsin and papain did not markedly affect the toxic or the serological activity of toxin in spite of a considerable liberation of free amino-acids. This finding will undoubtedly facilitate the production of specific antitoxin.

On the other hand, Jawetz and Meyer (1944) suggested that the toxicity may be associated with the enzyme make-up of the organism. Rechenmacher (1949) found greater catalase activity in the virulent organism than in the avirulent. A group of workers under Dr. Shrivastava (Sagar *et al.* 1956; Saxena *et al.* 1957; Srikantan *et al.* 1957 and 1958) also studied (1) deamination of amino-acids, (2) alkaline phosphatase activity, (3) oxidation metabolism, (4) transamination reaction, and (5) dehydrogenase in both virulent and
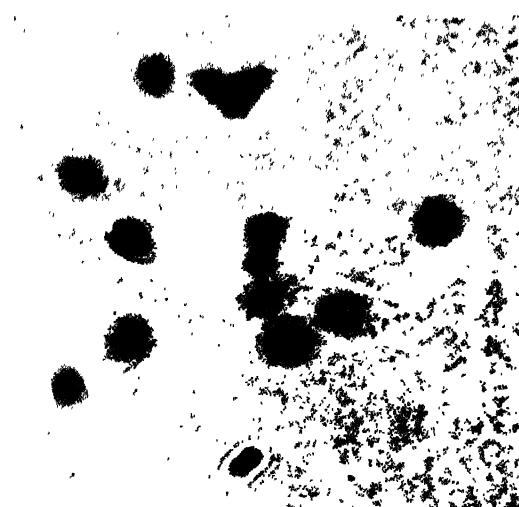
Photograph of stained *P. pestis* showing both
capsule and envelope.

avirulent strains of *P. pestis*. In addition they have studied the action of certain antibiotics and sulfa-drugs on the oxidative metabolism and transaminase reactions. The optimum temperature for these studies was 37·5° C. The results, however, indicate that although the enzyme studies did not so far give any clue to the difference between virulence and avirulence these might yield other interesting results.

Chemical antigenic structure

The work of the earlier workers (Lustig and Galleoti 1900; Rowland 1910, 1914; Brooks 1912; Morison *et al.* 1924) sufficiently indicated that the plague bacillus was composed of at least two varieties of proteins—one was soluble in distilled water or saline and contained the immunizing and the toxic substance and the other was insoluble in water and salt solution without any specific immunizing properties. Following the early attempts of Shrivastava (1939), Seal (1943, 1950, 1951*d*, 1953, 1954*a*), Baker *et al.* (1947, 1952), Amies (1951) and Bhagavan *et al.* (1955) have greatly advanced our knowledge on the chemical antigenic structures of the organism, using different methods of isolation and purification.

The author (1951*e*) succeeded in isolating a polysaccharide yielding osagone resembling that of Arabinose (melting point 166–168° C.) from the supernatant of the Haffkine plague vaccine as well as from the specific soluble protein and, to a lesser extent, from the bacterial debris of both virulent and avirulent protective strains. It was found absent in non-protective avirulent plague and pseudotuberculosis organism. The author therefore concluded that the protective substance of plague bacillus was a polysaccharide-protein complex probably a nucleo-protein. On the other hand, Korobkova and her colleagues (1951) are reported to have isolated two different apparently impure polysaccharides in *P. pestis*, reacting slowly with both antiplague and anti-pseudotuberculosis sera.

Without going into the details of the various fractions isolated it may be stated that in practice there is a good amount of uniformity in the findings of the above workers on the basic antigenic structure of plague bacillus and its dissociants in spite of the apparent diversity. Probably the differences noted were due mainly to the materials and methods used. The PD's (protective dose) of the fractions A and B of Bhagavan *et al.* were 8 or 8·3 micrograms against a challenge dose of 160 mld's, that of Baker *et al.* 12 to 22 micrograms against 100 mld's and that of Seal 0·6 to 2·5 micrograms against 13 mld's. The author, however, recently retested the antigen A and Baker *et al.*'s antigen (combined IA and IB) against a challenge dose of 5,000 virulent organisms (*P. pestis* 195/P) in *R. rattus* and *B. bengalensis* caught in Calcutta. The results are given in Table VIII.

TABLE VIII

Results of immunization of rats with different protein fractions of P. pestis

Antigen	Immunizing dose	Survivals in immunized	
		R. rattus	*B. bengalensis*
Antigen A of Seal ..	0·1 mg.	19/20	18/20
Antigen 1 of Baker *et al.*	0·1 mg.	16/20	14/20
Antiplague vaccine in enriched CH broth 1,000 millia organisms/ml. ..	0·4 mg.	10/20	9/20

N.B.—Given in two doses at weekly intervals; challenge dose
organisms (195/P.)

It appears that fractions A of Seal, IA and IB of Baker *et al.*, A of Bhagavan, etc., and the fraction I of Amies are all obtained from the envelope and/or capsular antigen of *P. pestis* as all of them have suggested. All these fractions are equally specific and highly protective against virulent plague infections. According to Baker *et al.* Seal's antigen may be a mixture of their IA and IB fractions, IA having the carbohydrate moiety as in Seal's antigen and IB and Amies' fraction being same as IA but without carbohydrate moiety.

In the words of Amies, not much importance need be given to the apparent discrepancies but what is needed is to evolve a standard technique for mass production of this specific antigen for human immunization.

Serodiagnostic procedures

The problem of serodiagnosis of plague infection in man or animal was fraught with difficulties for three main reasons, viz. (1) plague strains often formed unstable suspension in normal saline, (2) the antiplague serum raised against the whole organism acted against both plague and pseudotuberculosis organism, and (3) agglutination titre of the same organism differed at different temperatures of incubation. The author reinvestigated this problem in 1941-42 and evolved a technique by which all the difficulties were overcome. The agglutination is done with live suspension of organism grown on 5 per cent rabbit blood agar for 24–48 hours at 37° C. overnight. Two types of reactions occur: (1) floccular or woolly, and (2) granular, also described by Jawetz and Meyer (1944). The former is related to the envelope or protective antigen and gives low titre and the latter which is related to somatic antigen gives high titre agglutination. By using different antisera including the one against

the specific protein fraction A, absorbed or unabsorbed, the author (Seal 1951b) worked out the serological relationship between the different strains as given in Table IX.

TABLE IX

Serological relationship between P. pestis *and* P. pseudotuberculosis

Antisera produced against	P. pestis virulent and av. protective	Av. non-protective	P. pseudo-tuberculosis
Virulent P. pestis ..	+	+	+
Virulent P. pestis and absorbed with P. pseudotuberculosis	+	0	0
P. pestis boiled for ½ hour ..	0	+	+
P. pseudotuberculosis ..	0	+	+
Water-extractable protein ..	+	0	0
P. pseudotuberculosis and absorbed with P. pestis boiled	0	0	+

(a) *Serological test*

This serological test can also be used with fairly reliable result for the retrospective diagnosis of human cases of plague. The results of agglutination test in bacteriologically positive and suspected human cases of plague as carried out by the author in 1949 are given in Table X. For diagnosis of human plague cases Panja and Gupta (1948, 1949) used slide agglutination

TABLE X

Results of agglutination test in bacteriologically positive and suspected human cases of plague in 1949

Nature of cases	Number tested	Date of collection after onset	Date of examination after onset	Number positive	Per cent positive	Agglutination titre
Bact. ..	26	7–40 days	26–133 days	23 (3 doubtful)	88·4	1/10 to 1/200
Suspected cases (bact. negative or not done)	56	6–36 days	24–134 days	5	9·0	1/10 to 1/100
Non-plague cases	55	—	—	2 (both inoculated)	3·6	1/25 and 1/50

N.B.—The above results have been obtained in spite of sulphonamide and streptomycin treatment.

test with serum dilution of 1 : 3 or 1 : 4, usually on the seventh day after onset. But for earlier diagnosis bubo puncture fluid and sometimes blood culleni yield quicker result.

(b) Precipitation

The precipitation test could not be made popular for want of proper antigen. The isolation of the specific protein fractions by various workers described earlier opened the prospect of utilizing this technique as well for (1) serodiagnosis of plague infection in human beings and rats, (2) identification of plague strains, and (3) quantitative estimation of potency of antiplague serum. These methods have been actually utilized for plague studies (Seal 1951c, 1954a). For quicker diagnosis *ring precipitation* test may be done with patient's 1 : 2 to 1 : 10 sera against 1/1,000 dilution of Antigen A. Similarly, an unknown organism can be tested by growing the organism in CH broth and testing the filtrate against known antisera by the ring precipitation method. For qualitative precipitation the tube containing the mixture is incubated at 37° C. for 2 hours and then left in the refrigerator overnight. For quantitative test all the operations are carried out at 3–4° C. and incubated at that temperature for 24–48 hours (Seal 1954a).

(c) Complement fixation test

The earlier investigators Moses (1909), Damperoff (1910), Joltrain (1920), Simond (1898), Dickie (1926), Mitin (1938), and Wats *et al.* (1939) employed bacillar suspension or extracts as antigen but the results were not wholly satisfactory. The isolation of specific soluble protein fractions by the author and others greatly facilitated the performance of the complement fixation test using this antigen and high titre antiserum produced in rabbits against this antigen and it has been possible for the author (Seal 1953) and Chen *et al.* (1952) to (1) determine the evidence of plague infection in the tissue extracts of animals died of suspected plague, and (2) detect antibodies to fraction A or I in the sera of human convalescents and of immunized man and animal.

It may also be useful in the field diagnosis of wild rodent plague especially when the isolation of *P. pestis* or the interpretation of the pathological lesion at autopsy is rendered impossible by contamination or decomposition. Using this technique it has also been possible to differentiate between *P. pestis* and *P. pseudotuberculosis* and to detect antigenic deterioration of plague strains by cross-complement fixation test.

(d) Flocculation

Advantage has also been taken of the specific plague proteins in developing a flocculation technique for estimating the potency of antiplague

22B

serum (Seal 1947). Based on the constant serum and variable antigen method between Antigen A and the various horse and rabbit antiplague sera the author succeeded in establishing a flocculation test giving results in 5–15 minutes at 45° C. This technique may be suitable for estimating the potency of antitoxin raised against Ajl *et al.*'s plague toxin.

TREATMENT OF PLAGUE

(a) Serotherapy

The only treatment before the advent of chemotherapy with sulfa-drugs worth mention was the use of antiplague serum and the iodine solution. In the earlier years Choksey (1900) reported 60 per cent success in non-septicaemic cases. Considerable difficulty was encountered in producing a potent and effective antiplague serum. Following Naidu and Mackie (1931), Sokhey greatly improved the production of antiplague horse serum between 1936 and 1939 at the Haffkine Institute, but by that time sulfa-drug was about to come. Sokhey also developed a standard method of assay of this serum in white mice (Sokhey and Maurice 1935).

The most effective antiplague sera available recently are the ones produced against avirulent EV Madagascar strain and the Haffkine Institute antiplague serum. These have been used on a fairly large scale with better results. Girard (1941) and Le Gall (1943) reported about 60–65 per cent cure with EV antiplague serum and Sokhey and his collaborators, 76·5 per cent overall cure in 157 cases but taking only 71 bacteriaemic cases the rate of cure was only 49·3 per cent while only one death was registered in the remaining 86 cases. The pooled experience of the recent uses of antiplague serum as summarized by Meyer *et al.* (1952) is given in Table XI.

TABLE XI

Summary of the reported results of treatment with antiplague serum

Area or place	Serum-treated cases		Serum-untreated cases	
	Number	Fatality rate	Number	Fatality rate
Eastern Hemisphere (India, Indonesia, Japan, Middle East, Africa and Madagascar)	3,840	45·13	1,726	83·5
Western Hemisphere (North America, Argentina, Brazil and Peru)	19,540	32·75	213	74·07
Haffkine Institute (1911–43)	320 (71 bacteriaemic cases)	25·0 (50·7)	135	63·7

It is now considered that serotherapy with antitoxin might be more useful than the antiplague serum and that serum raised in rabbits is more potent than that raised in horses (Korobkova 1937; Meyer 1947; Seal 1954b). Recently Semerova et al. (1957) and particularly Khundanov et al. (1958) found the gamma-globulin fraction of antiplague serum to be more efficacious than the original antiplague serum as well as its beta-globulin and pooled globulin fractions in guineapigs.

(b) Chemotherapy

The earliest compound tried was prontensil in 1938 by Carman (1938) and Vine (1928) and sulfanilamide by Van Hoof. Schutze (1932, 1939) found sulfapyridine better than sulfanilamide. Soon sulfathiazole was discovered followed successively by sulfadiazine, sultamerazine and sulfamezathine. All were used for the treatment of plague in their turn. The combined experience of all workers including that of Sokhey, Wagle and others is given in Table XII. Of all the sulfa-drugs sulfamerazine seems to be superior to even sulfadiazine which has been more extensively used.

(c) Antibiotic treatment

Meyer and Quan first tried streptomycin in plague-infected animals in 1944. It seems Videalla tried it first in human cases in 1946. Comparative field trial with streptomycin was actually started in India in 1948. The rate of cure varied between 80 and 100 per cent. The total cases treated between 1945 and 1953 were 786 with only 33 deaths, a cure ratio of 95·8 per cent. These cases included septicaemic and meningeal plague also. The other antibiotics tried were aureomycin, chloramphenical, terramycin, neomycin and viomycin, etc., with good results, neomycin giving the best promise. Penicillin has also been used to check secondary infections. In the treatment of bubonic cases, however, sulfa-drugs may be enough. Very recently Semerova et al. (1957) used bacteriomycin with nearly as good result as streptomycin in plague-infected guineapigs.

TREATMENT OF PRIMARY PNEUMONIC CASES

Streptomycin had more striking results than sulfadiazine. Sometimes streptomycin with sulfa-drugs and antiserum have been tried with equal success in serious cases.

From the above records it can now be safely stated that effective treatment has been found in all types of plague cases including the primary pneumonic type. Thus the dread of plague has now been nearly completely removed.

TABLE XII

The combined results of treatment with sulfa-drugs (Indian and American data)

Drugs	Sokhey and Wagle (1941-49)		Simeons and Chittre (1946-47)		Datta Gupta (1948)		Combined results (India)		Meyer et al. (1952)	
	Cases	Per cent survived	Cases	Per cent survived	Cases	Per cent survived	Cases	Per cent survived	Cases	Per cent survived
Sulfanilamide	—	—	—	—	—	—	—	—	125	68·0
Sulfapyridine	122	73·0	—	—	—	—	122	73·0	725	45·4
Sulfathiazole	345	77·1	142	81·7	—	—	437	78·4	769	79·5
Sulfadiazine	168	91·5	704	82·0	41	90·1	1,097	83·8	1,061	91·4
Sulfamiazine	149	92·1	700	86·0	—	—	849	87·4	72	91·7
Sulfamezathine	—	—	—	—	37	89·2	37	89·2	—	—
Antiplague serum	157	76·5	—	—	—	—	157	76·5	—	—
Iodine soluble	149	46·3	—	—	—	—	149	46·3	—	—
Combined sulfathiazole and antiserum	60	80·0	—	—	—	—	60	80·0	—	—

Chemoprophylaxis

The effectiveness of sulfa-drugs in the treatment of plague cases permitted those drugs to be used as prophylaxis with a great deal of success even in the contracts of pneumonic cases (Seal 1949a).

The prophylactic vaccines

Antiplague vaccine prepared by Haffkine in 1897 is one of the earliest antibacterial vaccines for prophylactic use in human beings. This was prepared at a time when the knowledge about the organism, its culture medium, the growth factors and the antigenic structure was not fully known. With the improvement of such knowledge considerable improvement has been effected in the vaccine preparation and in the assessment of its potency. Although statistical evaluation of organized field trials are not many and there are other drawbacks which need more careful attention to bring about further improvement in the quality of vaccine, there are sufficient evidence and field records to show that vaccination against plague is of definite value in the prevention of plague (Simpson 1905 ; Dieudonne and Otto 1928; Otten 1936, 1940, 1941 ; Girard and Robic 1936; Girard 1946; Patel and Robello 1948; Meyer 1948, 1953).

The varieties of vaccine which have been used from time to time are :

    (1) Killed plague vaccine :

        (*a*) Haffkine broth vaccine,

        (*b*) Casein hydrolysate direct vaccine, and

        (*c*) Agar-grown vaccine.

    (2) Live avirulent plague vaccine (Otten, Girard and Robic).

    (3) Specific protein of plague organism—chemically prepared extracted.

    (4) Other killed vaccines tried :*

        (*a*) Pseudotuberculosis vaccine (Boyé 1932; Thal 1955),

        (*b*) Lipo-vaccine (Boyé 1933; Pons and Advier 1933),

        (*c*) Sugar vaccine (Minervin *et al.* 1935; Korobkova 1940),

        (*d*) Precipitated vaccine (alcohol and alum pptd.) (Wayson *et al.* 1946), and

        (*e*) In oil adjuvant (Spivack and Karler 1958).

Killed vaccine

The conditions precedent to the preparation of killed vaccine are the selection of a highly virulent plague organism and a suitable medium. Though toxicity and potency of vaccine was considered interrelated the recent work

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* None of these vaccines has been finally used for regular human immunization.

of Sokhey and Habbu (1945) as given in Table XIII shows that they are not, so far as the mouse protection is concerned. A standard method of biological assay of plague vaccine has been worked out by Sokhey (1947).

TABLE XIII

Toxicity test (LD_{50}) of different antiplague vaccines

Nature of vaccine	Toxic dose (ml.)	Mouse-pro-tective dose (ml.)
Haffkine broth vaccine (4 weeks' growth at 28° C.)	0·2	0·0052
Agar vaccine (37° C.) 1,000 million/ml.	1·0	0·004
Heated (54° C.) phenolized (0·5 per cent) broth vaccine	0·2	0·006
Formalin (0·05 per cent) killed and preserved by phenyl mercuric nitrate 1 mg./100 ml. broth vaccine	0·4	0·006
Casein hydrolysate direct vaccine (killed by formalin 0·07 per cent, 1·5 per cent phenyl mercuric nitrate)	0·6–0·8	0·004

According to the above findings agar-grown vaccine is least toxic and next best is the casein hydrolysate direct broth vaccine incubated at 32° C. now being used in India. It is practically free from non-specific protein, less toxic, more potent and less costly. One more advantage of the liquid medium is the elaboration of toxin converted into toxoid by formalin which may be helpful in raising the immunity against the possible toxin as well. The author, however, thinks that there is still some scope for improvement of both broth and agar-grown vaccine, particularly in respect of better medium for growth. There is also a lot of variation in the quantum of organisms included per ml. of the vaccine. In any case, according to Meyer (1953) none of the vaccines, given in two doses, contains enough specific antigen to produce fully-effective immunity. Experiments carried out in human volunteers show that the quantity of vaccine which will contain 2-3 mg. of fraction I is capable of favourably altering the susceptibility of 50 per cent of inoculated persons. If this is to be achieved with killed vaccine reinoculation at intervals of 3–6 months is essential.

Live avirulent vaccine

Girard in Madagascar with avirulent EV strain and Otten in Java with avirulent Tiwidej strain obtained great success with live vaccine. Similar

vaccine has also been used in Argentina, Belgian Congo, Brazil, French West Africa, Tunisia and Union of South Africa (Pollitzer 1954). But Sokhey and his coworkers in India did not agree to try such vaccines due to certain amount of risk involved such as the possibility of changing into virulent form. Besides, greater control and supervision for its preparation and better storing facilities are necessary. Also, without freeze-drying, it is not possible to keep the live vaccine for its use in distant parts of this tropical country.

Specific soluble protein for human immunization

The author (1943, 1951b, 1953) produced experimental evidence to show that the immunizing substance in the Haffkine plague vaccine is a specific soluble protein and the potency of the vaccine depends entirely on this specific substance. According to Meyer 2-3 mg. of this specific antigen is capable of producing a high protective immunity in man. The possibility of its use in human immunization against plague infection needs to be explored. Even the bacteria-free filtrate of casein hydrolysate broth vaccine may be dried and the crude protein may be used in quantities required for producing sufficient immunity. The present status of therapy of plague including the prophylactic vaccines has been thoroughly reviewed by the author (Seal 1960c).

CONTROL MEASURES

Control measures can be divided into four main sections, viz. (1) control of plague in human beings, (2) control of rodents, (3) control of vectors, and (4) control of spread of plague at distance.

As already indicated considerable improvement has already been effected in the prevention and control of plague in human beings due to the discovery of effective drugs, prophylactic vaccines, therapeutic antisera and disinsectization methods. Rat-proofing of houses and godowns, control of movement of grains and of patients and contacts are adopted to stop spread of plague. The most important discovery which has directly influenced the control of plague is that of insecticide like DDT, BHC and DIELDRIN, etc. In India, the vector control has been effected not only through the dusting of burrows and the affected houses with 10 per cent DDT powder but the extensive antimalarial operation with wettable DDT suspension and emulsion seems to have given the collateral benefit of killing the fleas in the houses. Among the other insecticides chlordane, organic phosphates, pipernyl compounds and fumigants like HCN, cyanogas, etc., are also used. A study on the comparative pulicidal values of cyanogas, DDT and BHC by Wagle and Seal (1953) has shown that DDT is more efficient and economical insecticide among those so far as the common Indian vector fleas are concerned. Dieldrin

has also been found to be equally effective. The flea indices in different parts of India are showing a definite tendency towards decrease, the *cheopis* index hardly rising above 0·5 and this factor combined with that of herd resistance in the commensal rat mentioned earlier has been partly responsible for the gradual reduction of plague in India.

Resistance in flea population

Since several instances of development of resistance in flea population have recently been detected in India (in Mysore and Bombay States), the proposition should be met by an immediate change and intensive use of insecticide and a close vigilance kept in other areas so that the necessary steps may be taken on the slightest suspicion of resistance being developed in the flea population. Application of DDT in smaller doses than necessary for their control may ultimately prove to be dangerous and uneconomical. Although the present situation could not be avoided as antimalaria campaign had to run its own course, if antiflea measures are to be undertaken it should be done on a thorough basis with proper dose of the insecticide and should be spread over all the year round instead of depending upon the two or three rounds of DDT as a part of antimalaria operation. According to the recent work of the author and of Baltazard (1960) the reservoir of infection being the rodents, wild as well as commensals, the essential step needed for the eradication of plague is the systematic destruction of these rodents as has been done in U.S.S.R. (Fenyuk 1960). But so long as this is not possible due to financial reasons the main target of attack should remain with the vector fleas and wherever possible destruction of rats.

For the control of rodents, trapping, baiting, poisoning and various fumigants are in use. Among the fumigants, HCN, CO_2, CS_2, chlorpicrin and $Ca(CN)_2.SO_2$ have been successfully used. For poison-baiting a variable amount of success has been attained by using $BaCO_3$, arsenic preparations, phosphorus, red squill, zinc phosphate, antu, sodium fluoracetate and some anticoagulants. Virus and fungus infections have also been suggested. In fact, Russia seems to have launched a total eradication campaign against rodents as a whole and particularly against the wild rodents. According to the observations made by the author in Calcutta (Bhattacharji and Seal 1954) the normal range of movement of rats being 200 yards intensive antirat and anti-flea measures around 200 yards of the actual ratfall may be sufficient to control the spread provided the actions are promptly taken. As a long-term measure, however, slums, huts, and godowns which harbour rats should be replaced by pucca buildings or structures which would prevent rat harbourage. The success of control measures will depend upon the extent of improvement brought about in this respect.

CONCLUDING REMARKS

With the natural decline of plague either through the development of herd resistance in the commensal rats or by active antiplague measures it seems that the infection tried to recede to its original host reservoirs, namely the wild rodents, and it may be that the same phenomenon will follow in India, too, as recently noted by Baltazard *et al.* (1958). The author has already discussed about the epidemic behaviour of plague from the historical standpoint. It may now be stated that, although plague has often shown decline even when left alone, it flared up again in due course. But before a general recrudescence occurs a change or mutation takes place in the organism facing extinction as a natural process for survival. Thus the three varieties of plague organisms described by Devignat may be related to such changes prior to the three successive pandemics, and one never knows when the history will repeat itself. There is nothing therefore to be complacent about the disease, in spite of the great decline in the incidence at present. Rather this is the time to make certain fundamental studies on the evolution of plague in the fields and to prepare our weapons for better control of the situation which may yet arise under the secular behaviour of this fell disease. At the same time there seems to be no reason to fear as widespread outbursts are not likely to occur in the face of the knowledge and experiences gained about its treatment, prevention and control during the last half of a century. Nevertheless, from both national and international points of view priority should be given to the detection and elimination of foci of infection among the rodents, either commensal or wild. But so long as such a procedure does not become feasible, both antiflea and antirat measures should be continued in the places where plague either in rodents or in man has been recently reported and strict vigilance should be kept for any such incidence from any quarter, however small it might be. The vigilance should also include regular examination of rodents from different areas for possible infection and resistance against it and of flea population for resistance against the currently used insecticides, in addition to the strict observance of quarantine regulations.

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APPENDIX I

The chronological order of the development of knowledge about plague

1894 (14 June)	Discovery of *Pasteurella pestis* by Yersin and Kitasato from the blood, bubo and spleen of human patients.
1895	Antiplague serum by Yersin, Calmette and Borrel.
1896	Antiplague vaccine by Haffkine at Bombay.
1897 Ogata (Formosa) 1898 Simond (India)	Fleas as vectors of transmission from rat to rat and man.
1902-3	Gauthier and Raybaud proved transmission by fleas experimentally.
1904	Dürk of Austrian Plague Commission worked out the pathological anatomy of plague.
1905	Glen Liston (Bombay) and the Indian Plague Research Commission confirmed rat-flea-rat transmission.
1910	Rowland postulated endotoxin of plague bacillus.
1914	Bacot and Martin (Indian Plague Research Commission) described the role of blocked flea in the transmission of plague infection.
1928	Jorge (Africa) described plague in wild rodents (Sylvatic plague).
1930	Naidu et al. utilized horses at Bombay for the production of antiserum, the only available remedy till that time.
1932	Schutze differentiated the envelope and somatic antigen.
1932-35	Sokhey at Bombay described mouse-protection test for the standardization of anti-plague vaccine and sera.
1935	Otten in Java introduced live attenuated plague vaccine followed by Girard and Robic in Madagascar and Pirie and Grasset in South Africa in 1938.
1939	Sokhey standardized the virulence test, culture medium and described optimum conditions of growth of plague bacillus.
1939	Schutze (Great Britain) and Sokhey (India) introduced treatment of plague with sulfa-drugs.

1940	Bhatnagar (Kasauli, India) classified the plague and pseudotuberculosis organisms by improvised agglutination test.
1940-41	Seal and Mukherji at Bombay utilized modified casein hydrolysate medium for the growth of plague strains and the study of chemical antigenic structure. Seal also solved the problem of auto-agglutination of plague strains by using live organisms against the various types of antisera raised, and developed serological test for identification of virulent plague from non-protective plague and pseudotuberculosis strains.
1943	Seal isolated specific soluble protein fractia of the plague bacillus and also the fraction correlated with *P. pseudotuberculosis*.
1944	Jawetz and Meyer (U.S.A.) differentiated virulence from toxicity of plague bacillus and studied the mechanism of immunity in plague.
1946-47	Viswanathan (Bombay) used DDT as an effective insecticide in India.
1947	Baker *et al.* (U.S.A.) purified specific protein antigen of plague bacillus.
1947-48	Streptomycin was used very successfully in both bubonic and pneumonic plague in India and other places.
1949	Devignat (Belgian Congo) classified plague strains into *orientalis, antiqua* and *mediavelis* types on the basis of reaction on glycerine and production of nitrous acid.
1950	Sokhey *et al.* (Bombay) adopted casein hydrolysate medium for the preparation of antiplague vaccine.
1956	Burrows and Bacon described two additional antigens, V and W, in plague bacillus.
1952-57	Seal proved on the basis of experimental epidemiology that partially immune rodents act as reservoirs of infection during inter-epidemic period by harbouring plague organisms of reduced virulence in an inapparent form or in chronic foci in spleen and not by fleas under the Indian environmental conditions.
1959	Seal presented evidence of both capsule and envelope being present in virulent plague bacillus.